ISCoS The International Spinal Cord Society

www.nature.com/sc

ARTICLE

Check for updates

Safety and feasibility of autologous olfactory ensheathing cell and bone marrow mesenchymal stem cell co-transplantation in chronic human spinal cord injury: a clinical trial

Homa Zamani[1], Mina Soufizomorrod[1]✉, Saeed Oraee-Yazdani[2]✉, Dariush Naviafar[3], Mohammadhosein Akhlaghpasand[2], Afsoun Seddighi[2] and Masoud Soleimani[1,4]✉

STUDY DESIGN: This is a phase I clinical trial.
OBJECTIVES: Our objective was to assess the safety and feasibility of autologous mucosal olfactory ensheathing cell (OEC) and bone marrow mesenchymal stem cell (MSC) co-transplantation in people with chronic, complete (American Spinal Injury Association (ASIA) Impairment Scale (AIS) classification A) spinal cord injury (SCI).
SETTING: This study was performed at Shohada Tajrish Hospital, Tehran, Iran.
METHODS: Three individuals with the traumatic SCI of the thoracic level were enrolled. They received the autologous OEC and MSC combination through the lumbar puncture. All adverse events and possible functional outcomes were documented performing pre- and post-operative general clinical examination, magnetic resonance imaging (MRI), neurological assessment based on the International Standard of Neurological Classification for SCI, and functional evaluation using Spinal Cord Independence Measure version III (SCIM III).
RESULTS: No serious safety issue was recorded during the 2 years of follow-up. MRI findings remained unchanged with no neoplastic tissue formation. AIS improved from A to B in one of the participants. SCIM III evaluation also showed some degrees of progress in this participant's functional ability. The two other research participants had negligible or no improvement in their sensory scores without any changes in the AIS and SCIM III scores. No motor recovery was observed in any of the participants.
CONCLUSIONS: Overall, this 2-year trial was not associated with any adverse findings, which may suggest the safety of autologous OEC and bone marrow MSC combination for the treatment of human SCI.

Spinal Cord (2022) 60:63–70; https://doi.org/10.1038/s41393-021-00687-5

INTRODUCTION

The limited regenerative capacity of the spinal cord often confronts people affected by the spinal cord injury (SCI) with permanent disability and dependency [1]. It has been a while that cell replacement therapies have emerged as promising strategies for SCI treatment, among them olfactory ensheathing cells (OECs) and mesenchymal stem cells (MSCs) are considered as two of the most encouraging candidates [2].

OECs are a particular type of macroglia residing in both peripheral (olfactory mucosa and olfactory nerve) and central (olfactory bulb) parts of the olfactory system and support the continuous neurogenesis of the olfactory neurons throughout life [3]. The high regenerative potential of OECs like the stimulation of axonal regrowth, re-myelination, and guidance across the lesion is the rationale behind the extensive use of these cells in the experimental models of SCI [4, 5].

Bone marrow MSCs also possess different regenerative qualities that make them beneficial for spinal cord repair. They can differentiate into various cell types and secrete trophic factors that promote axonal growth while lacking tumorigenicity. Besides, through their immunomodulatory, anti-inflammatory, and anti-apoptotic effects, MSCs can play a vital neuroprotective role within the injured tissue and provide axons with a permissive environment for their regeneration [6].

Many clinical trials have been conducted to assess the safety and functional recovery following OEC or MSC transplantation alone in people with traumatic SCI [7, 8]. However, concerning the progressive, multifactorial nature of this injury, it seems that applying an appropriate combination of such highly potent cells may lead to better outcomes [9]. The safety and efficacy of some combination therapies have been documented in preclinical models of SCI [10]. Several clinical studies have also been designed to demonstrate the feasibility of this approach for spinal cord regeneration [11, 12]. In the present study, we evaluated the safety and feasibility of autologous mucosal OEC and bone marrow MSC co-transplantation into individuals with chronic, complete, traumatic SCI.

[1]Department of Cell Therapy and Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. [2]Functional Neurosurgery Research Center, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Department of Neurosurgery, Erfan Hospital, Tehran, Iran. [4]Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ✉email: m.soufi@modares.ac.ir; saeed_o_yazdani@sbmu.ac.ir; soleim_m@modares.ac.ir

Received: 19 January 2021 Revised: 26 July 2021 Accepted: 28 July 2021
Published online: 9 September 2021

METHODS

Study design

This study was registered at the Iranian Registry of Clinical Trials (registration no. IRCT20160110025930N2). All of the procedures were performed after a written informed consent including the detailed description of all of the experimental processes, the probable adverse events, and the potential for no benefit was obtained from each of the research participants. Inclusion criteria were having a thoracic SCI, complete loss of sensory and motor function below the site of injury (American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade A), at least 6 months post injury (chronic phase), male or female aged 18–70 years, and suffering from no mental disturbance. Exclusion criteria included having severe medical complications or other lesions of the nervous system, spinal stenosis or compression, severe muscle atrophy, and clinically significant chronic sinusitis or polyps of nasal cavities.

All of the participants went through a regular rehabilitation program started 6 months before the operation and continued until discharge. This scheduled program included physical therapy strategies with a great focus on overground and treadmill locomotor training activities. More details about the performed rehabilitation protocol can be found in Supplementary File 1.

Olfactory mucosa biopsy, bone marrow aspiration, and cell isolation

To obtain mucosal biopsies under general anesthesia, the participants were hospitalized. They were placed in a supine position in the operation room. After irrigating and disinfecting the nasal cavities, the biopsy was harvested from the area of superior turbinate using the endoscope. The collected specimens were placed in a cold Hanks' Balanced Salt Solution (HBSS, Sigma-Aldrich, St. Louis, USA) consisting of 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, Grand Island, USA) and transferred to the cell culture laboratory in a sterile, sealed container. All the next steps were carried out according to the protocol described by Tabakow et al. [7]. Briefly, the tissue fragments were digested with a 2.4 U/ml dispase II solution (Sigma-Aldrich). After removing the olfactory epithelium, the lamina propria was cut into small pieces and treated with a 5 mg/ml collagenase H solution (Sigma-Aldrich), followed by centrifugation and culturing of the cells.

Bone marrow (100 ml) was aspirated from the posterior superior iliac spine of the iliac crest during the same anesthesia. Based on our previously described method [11], MSC isolation was done using a 1:3 volume of Ficoll solution (1.077 g/l, Sigma-Aldrich). The biphasic prepared sample was centrifuged, and the mononuclear cell layer was separated carefully. After performing three washing steps with HBSS, these cells were also cultured in the appropriate culture conditions.

In-process and final quality control tests

Cell characterization. The characterization of OECs was performed using both S100 and glial fibrillary acidic protein (GFAP) immunocytochemical staining. The isolated cells were fixed and permeabilized with 4% paraformaldehyde and 0.1% Triton X-100 (both Sigma-Aldrich), respectively. The blocking step was done with 10% goat serum (Gibco), followed by incubation with anti-S100 or anti-GFAP (both Santa Cruz Biotechnology, CA, USA) antibody at 4 °C overnight. The samples were then incubated with a suitable horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich) for 1 h and finally exposed to 3, 3′-diaminobenzidine (Sigma-Aldrich) to produce the chromogenic reaction. Hematoxylin (Sigma-Aldrich) was applied for nuclear counter-staining, and cell visualization was carried out through a light microscope.

To confirm the identity of bone marrow-derived cells as MSCs, they were subjected to both flow cytometric analysis of verification markers and differentiation capacity toward adipogenic and osteoblastic lineages [11].

Sterility test and gram stain. The cells were regularly assessed microscopically to confirm their normal growth and the lack of visible contaminations. A direct inoculation sterility test was done every 5 days and also from the final harvested product [13]. In brief, a sample was taken from the supernatant of the cultured cell and injected into two microbial culture tubes containing Tryptic Soy Broth and Fluid Thioglycollate Medium (TSB and FTM, both Merck, Darmstadt, Germany). The test tubes were incubated for 14 days at 25 °C and 35 °C for TSB and FTM, respectively. Before the transplantation, the standard Gram staining was performed as well on the final cell suspension to verify the absence of contaminating organisms.

Viability assay. A propidium iodide (PI) stating was carried out on the final cell mixture before the operation. Following the sample preparation, 1×10^4 cells/100 µl were mixed with a 5–10 µl PI fluorescent agent (Sigma-Aldrich). The sample was then incubated in the dark for 1 min and finally analyzed by FACS Calibur flow cytometer and FlowJo software.

Cytogenetic analysis. The cytogenetic stability of the cultured cells was studied using the standard GTG-banding technique. The cells were delivered to the cytogenetic department of Children's Medical Center and harvested for the conventional karyotype examination.

Cell transplantation

The cells were separately trypsinized and mixed in a ratio of 1:1 of OEC: MSC in 2 ml injectable saline solution (0.9%) at a final concentration of 15×10^6 cells per ml. Under sterile conditions, the intrathecal injection of 30×10^6 cells was carried out into each participant according to our previous study [14]. Briefly, the participants were hospitalized and placed in the operating room in a lateral position. After aseptic preparation, the sample was slowly injected into the subarachnoid space of the L4/L5 level through the lumbar puncture using the spinal needle 24 G. The needle was kept in place for one additional minute to avoid leakage.

Pre- and post-operative evaluations

The research participants were monitored regularly to record the vital signs and any adverse events based on the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 guideline. The preoperative magnetic resonance imaging (MRI) scans were carefully analyzed and compared with the ones taken 12 and 24 months post surgery to track the radiological changes of the spinal cord and its surrounding tissue. To follow the neurological status and functional recovery of the participants, the International Standard of Neurological Classification for Spinal Cord Injury (ISNCSCI) sensory and motor scoring system and the Spinal Cord Independence Measure (SCIM) version III scale were respectively evaluated before and after surgery at 6-month intervals up to 24 months. An electromyography test was carried out when the individuals claimed any improvements in their motor activity to confirm that the reported muscle contraction is voluntary.

RESULTS

Participants' characteristics

Started in 2018, participant recruitment for this pilot study took over 4 months, during which 11 individuals volunteered. After the careful evaluation of the volunteers based on the pre-determined criteria, only three individuals were found eligible to enroll, all of whom had a chronic, thoracic lesion resulted either from a road traffic accident or falling from a height. The preoperative neurological evaluation of the research participants showed complete paralysis that was persistent over at least 6 months before the surgery. All three participants had been undergone spinal decompression and fixation surgery at the time of injury. Each participant enrolled in this trial and underwent cell transplantation at a different date since the beginning of the recruitment period and was followed up for at least 2 years until the end of the study (Table 1).

Cell culture and quality control tests

The cells isolated from the three participants were grown in culture for 3–4 weeks until they were ready for transplantation. They all were in a good growth state and had a normal and healthy appearance. The olfactory-derived cells were positive for both S100 and GFAP identification markers of OEC (Fig. 1a). The differentiation capacity of the cultured MSCs toward the osteoblastic and adipogenic lineages was also confirmed (Fig. 1b), and they were found to be positive for CD73, 90, and 105 and negative for CD34 and 45 cell surface antigens (Fig. 1c).

The visual inspection of in-process and final sterility tests did not show any turbidity indicating microbial growth throughout the culture. The Gram stain also did not detect the presence of microbial contaminants in the final product (data not shown).

Table 1. Demographic and clinical features of the research participants.

Follow-up (month)	AIS	Time from SCI (month)	Zone of partial preservation	Vertebral level of injury	Cause of injury	Age (year)	Sex	Participant number
27	A	52	T10	T10	Road traffic accident	27	M	1
25	A	23	T12	T11	Falling from height	66	F	2
24	A	75	T12	T12	Road traffic accident	26	M	3

M male, *F* female, *SCI* spinal cord injury, *AIS* ASIA Impairment Scale.

The preoperative PI viability assessment of the OEC/MSC mixture demonstrated at least 92% alive cells, which showed their potential suitability for transplantation (Fig. 1d). In addition, no numerical or structural chromosome abnormality was indicated in the cytogenetic analysis of the cells (Fig. 1e).

Safety assessment

Clinical examination. No mortality or severe adverse event occurred in the research participants within the follow-up period. No evidence of fever, hypersensitivity, inflammation, meningitis, or other allergic or infectious diseases that could be attributed to the cell transplantation was observed. None of the transplant recipients showed the deterioration of their neurological condition. Two of the participants (participants 2 and 3) reported either the onset or increase of neuropathic pain after transplantation that was alleviated by receiving gabapentin and vitamin B1. Appropriate antispasmodic drugs were prescribed for participant 2 with an increased spasm frequency in the lower extremity muscles. One month after transplantation, participant 1 had a bronchial infection that was not related to the performed intervention. Participants 2 and 3 also complained of a mild headache that started on the day following the mucosal biopsy and lasted for about 2 days. All three individuals experienced mild, temporary hyposmia that spontaneously resolved after 1–2 weeks. A summary of the observed adverse events is provided in Table 2.

Radiology. Some of the worst effects were detected on the visibility of images in all of the research participants who had been undergone spinal instrumentation. However, the radiological assessments did not reveal any changes in the spinal cord and its surrounding parenchyma with no evidence of neoplastic tissue formation. MRI findings also showed no excessive spinal compression (Fig. 2).

Evaluation of neurological, functional, and other subjective changes

The participants' neurological and functional status at enrollment remained unchanged until the time of surgery. No improvement or deterioration was seen in the sensory and motor function of participant 1 over the 2 years of observation. Participant 3, however, had a negligible improvement in his ASIA sensory scale with a 2-point increase in the light touch and pinprick sensation at both sites but no perianal sensation. The most promising result was observed in participant 2. She had significant improvement in her sensory score that was initially detected 6 months after transplantation and continued progressively. The sacral exam showed the presence of bilateral S4-5 and deep anal pressure sensation at this participant 1 year post surgery. The ASIA sensory score increased 9 points for each of light touch and pinprick scales at both sides and reached the total amount of 46 at the end of the study. She had changes in her lower extremity motor function, but no voluntary muscle contraction was recorded in the electromyography test of this participant (data not shown). Based on these results, the AIS classification of participant 2 altered from A to B. In contrast, participants 1 and 3 had no change in their AIS.

Monitoring the functional changes, using the SCIM III scale, also revealed no progress in the functional ability of participants 1 and 3. On the other hand, some abilities of participant 2 including self-care activities and bed mobility improved compared to the preoperative time. She gained more independence in her daily life with a 6-point elevation in her total SCIM III score (Table 3 and Supplementary File 2—Supplementary Figs. S1–S3).

The participants also reported some other subjective changes during the study. Two of them (participants 2 and 3) declared having urination sensation. Participant 2 reported a feeling of defecation as well. None of the individuals regained their complete bladder or bowel sphincter control. From the aspect of trunk movements, stability, and trunk equilibration in the sitting and standing positions, more improvement was reported by participants 2 and 3 (Table 4).

DISCUSSION

Many intrinsic and extrinsic growth inhibitory factors are involved in the progression of SCI and its lack of response to current treatments. It seems that implementing a combinatorial strategy would be more beneficial than a single therapy as it can address more aspects of the disease [9].

To date, different approaches have been applied to combine individual SCI treatment options. One of the recent avenues of research in this area has focused on the transplantation of regenerative cells together to repair different pathological barriers and also take advantage of the synergistic effects of these cells. The practicality of this new scenario using various cell combinations has been tested in some preclinical and clinical studies and reported the safety and some degrees of efficacy for them [12, 14, 15]. However, there is still a long way to discover the most efficient combinatory cell-based design for human SCI.

Because of their physical support, expressing some growth and guidance factors, phagocytic function, and having more efficiency for migration and integration with astrocytes than Schwann cells, OECs are increasingly taking into account as attractive tools for the repair of the injured spinal cord [5]. MSCs have also shown more benefits over other stem cell types since they are easily accessible from varied sources with no ethical issue. They have this capacity to highly proliferate and differentiate toward both neuronal and non-neuronal lineages while they are generally safe and less likely to produce tumors [16].

The combined application of MSC and OEC for SCI treatment has been previously examined in several veterinary trials. Deng et al. showed that compared to the singular therapeutic strategy, the co-grafting of human bone marrow stem cell and olfactory ensheathing glia to a thoracic SCI rat model was associated with better histologic, electrophysiologic, and functional outcomes [17]. Another study in 2015 also reported more functional recovery in rats treated with OEC/MSC combination than using either cell alone and justified it by the enhanced anti-apoptotic effect of this

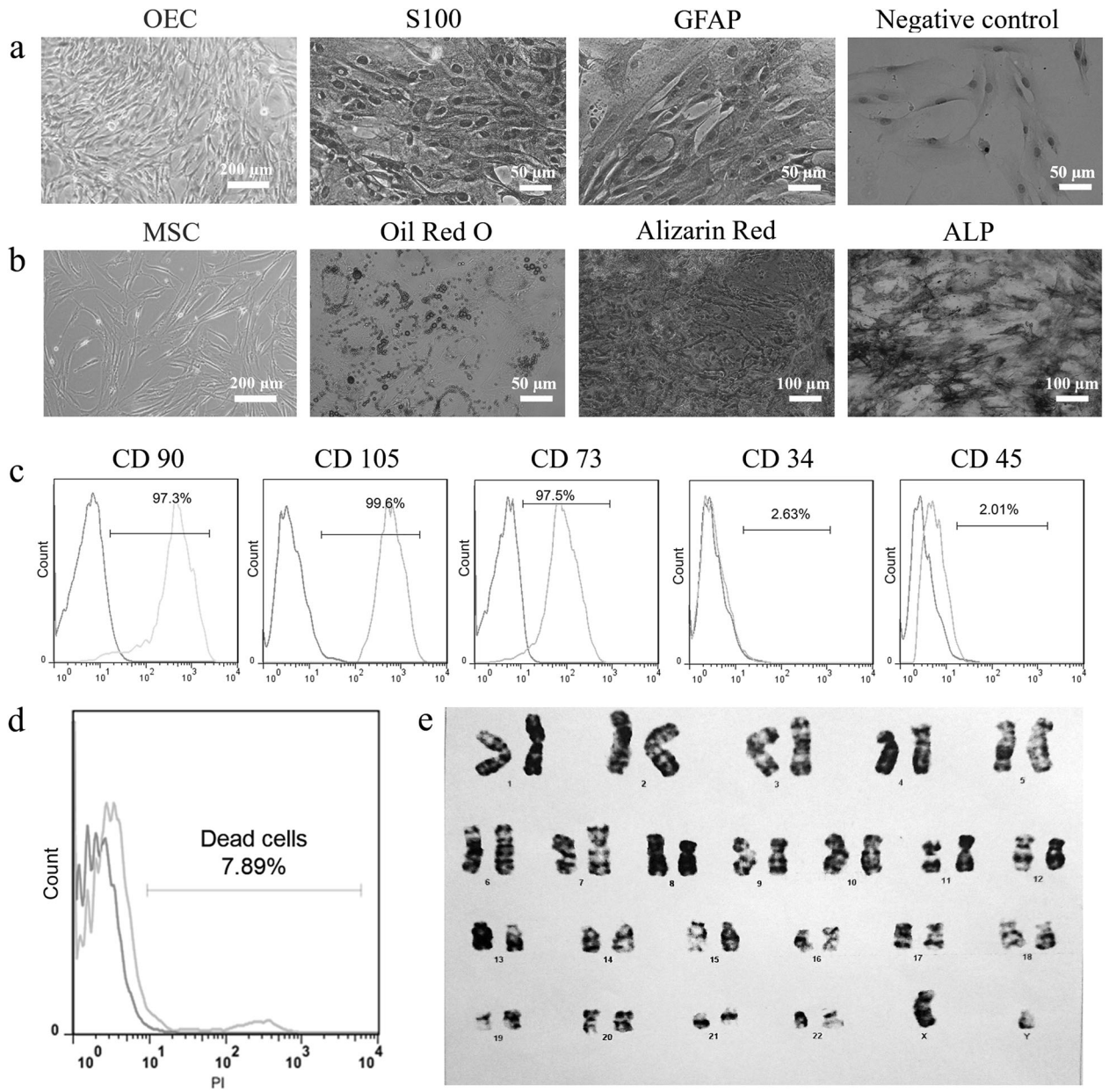

Fig. 1 The quality control test results of the cultured cells. a The cultured OECs exhibited a normal bi- or multipolar morphology with long processes. The ICC analysis of these cells using DAB showed positive brown staining for S100 and GFAP identification markers, and the specificity of the results was confirmed by a negative control. **b** MSCs preserved their adherent, fibroblast-like appearance during the culture. The adipogenic and osteoblastic differentiation capacity of the isolated cells was demonstrated through oil red o, alizarin red, and alkaline phosphatase staining. **c** The bone marrow-derived cells were positive for CD73, CD90, and CD105 and negative for CD34 and CD45 markers using flow cytometry, which is in line with MSC properties. **d** The PI viability assessment of the OEC/MSC mixture revealed 92% alive cells for transplantation. **e** No chromosomal anomaly was detected in the karyotype examination of the cells by the conventional GTG-banding method. ICC immunocytochemical staining, GFAP glial fibrillary acidic protein, DAB 3, 3′-diaminobenzidine, OEC olfactory ensheathing cell, MSC mesenchymal stem cell, PI propidium iodide, ALP alkaline phosphatase.

cell mixture [18]. Moreover, the co-administration of these cells into the SCI rats resulted in the restoration of the motor function and reduced the inflammatory cells in the injured area [19]. These encouraging results prompted us to investigate the translatability of this combination therapy into human SCI.

Both the olfactory bulb and olfactory mucosa are used to harvest OECs for autologous transplantation. However, mucosal OECs are considered the preferable type of these cells for human application as the isolation of bulbar OECs requires performing a more invasive surgery while the olfactory mucosa is easily accessible [20]. As a result, in this study, we used the olfactory mucosa as our source of OEC. Except for a mechanical and enzymatic dissociation step, the cells did not undergo any further purification. The reason was that according to some previous studies a heterogeneous population of OECs may have more potential for repairing different aspects of such neural defects [21].

We decided to use the lumbar intrathecal route of administration as our mode of cell delivery since it is considered a less invasive method than the direct parenchymal injection [22]. Besides, studies have shown that the cells injected into the cerebral spinal fluid by the lumbar puncture are better survived and delivered to the spread sites of injury [23]. More progress was

Table 2. The observed adverse events following the olfactory mucosa biopsy and cell transplantation based on the CTCAE version 5.0.

Participant number	Number of AE (% respect to total AE)	CTCAE term	System organ class	Occurrence (+) or exacerbation (↑) of AE	Causal link with OEC/MSC co-administration	Grade[a]
1	1 (16.6)	Bronchial infection	Infections and infestations	+	Not related	II
2	3 (50)	Headache	Nervous system disorders	+	Probable	I
		Neuralgia[b]	Nervous system disorders	↑	Probable	II
		Spasticity	Nervous system disorders	↑	Probable	II
3	2 (33.3)	Headache	Nervous system disorders	+	Probable	I
		Neuralgia	Nervous system disorders	+	Probable	I

CTCAE Common Terminology Criteria for Adverse Events, *AE* adverse event, *OEC* olfactory ensheathing cell, *MSC* mesenchymal stem cell.
[a]Severity of the AE. Grade I: mild; intervention not indicated. Grade II: moderate; minimal, local, or noninvasive intervention indicated.
[b]Neuropathic pain.

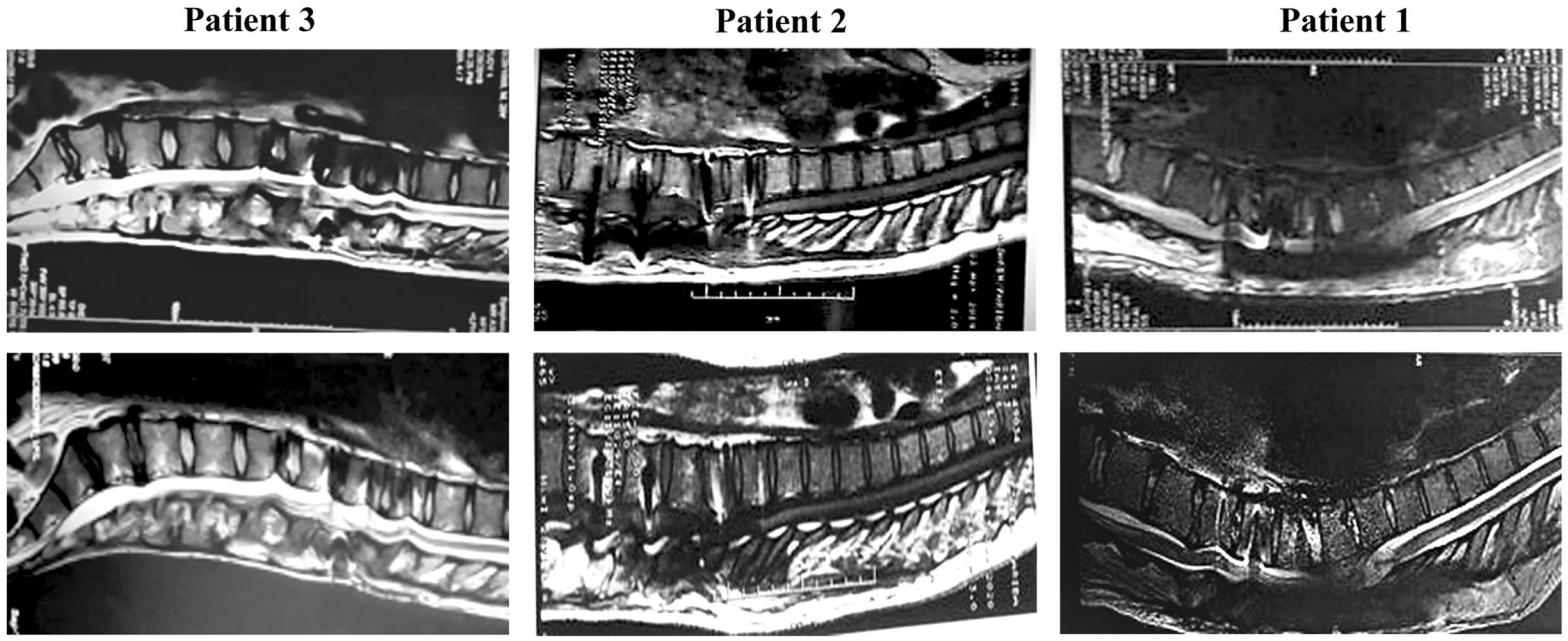

Fig. 2 MRI studies of the injured spinal cord before and 24 months after cell transplantation. No tumor tissue, pseudomeningocele, or syringomyelia was detected during the follow-up period. MRI magnetic resonance imaging.

Table 3. Detailed neurological and functional examinations of the participants before and after cell transplantation.

Variable name	6 months before transplantation	At the time of transplantation	6 months after transplantation	12 months after transplantation	18 months after transplantation	24 months after transplantation
Participant 1						
Sensory status (LTR or PPR)	33	33	33	33	33	33
Sensory status (LTL or PPL)	33	33	33	33	33	33
Motor status (LER)	0	0	0	0	0	0
Motor status (LEL)	0	0	0	0	0	0
DAP	N	N	N	N	N	N
VAC	N	N	N	N	N	N
AIS	A	A	A	A	A	A
SCIM	71	71	71	71	71	71
Participant 2						
Sensory status (LTR or PPR)	37	37	39	44	46	46
Sensory status (LTL or PPL)	37	37	39	44	46	46
Motor status (LER)	0	0	0	0	0	0
Motor status (LEL)	0	0	0	0	0	0
DAP	N	N	N	Y	Y	Y
VAC	N	N	N	N	N	N
AIS	A	A	A	B	B	B
SCIM	33	33	35	39	39	39
Participant 3						
Sensory status (LTR or PPR)	38	38	38	40	40	40
Sensory status (LTL or PPL)	38	38	38	40	40	40
Motor status (LER)	0	0	0	0	0	0
Motor status (LEL)	0	0	0	0	0	0
DAP	N	N	N	N	N	N
VAC	N	N	N	N	N	N
AIS	A	A	A	A	A	A
SCIM	80	80	80	80	80	80

LTR light touch (right), *LTL* light touch (left), *PPR* pinprick (right), *PPL* pinprick (left), *LER* lower extremity (right), *LEL* lower extremity (left), *DAP* deep anal pressure, *VAC* voluntary anal contraction, *AIS* ASIA Impairment Scale, *SCIM* Spinal Cord Independence Measure, *N* no, *Y* yes.

also achieved in the ISNCSCI scores of the research participants by injecting the Schwann cell and MSC through the cerebral spinal fluid than the direct injection into the injured area [11, 14].

Our primary outcome measure in the designation of this clinical study was to evaluate the safety of autologous mucosal OEC and bone marrow MSC co-transplantation in people with complete, chronic SCI. Altogether, no radiological or systemic adverse event was detected in any of the participants, which may suggest the safety of this combinational cell therapy approach for human SCI. All of the recorded negative findings were classified as mild (grade I) to moderate (grade II) in terms of severity. Headache and neuropathic pain were the most prevalent adverse events. The headache was transient and likely relevant to the biopsy from the nasal mucosa, although the previous experiments did not report such a negative event [24]. Two of the three participants experienced the initiation or intensification of neuropathic pain. Research studies show that in addition to the nerve injury, this medical condition can occur as a consequence of cell transplantation [25]. According to these studies, both single and combinatorial cell therapeutic interventions can increase the risk of neuropathic pain, and its incidence has been reported about one-third to one-half of the total number of transplant recipients [11, 14, 26]. However, due to the small sample size of our non-controlled survey, we cannot make any conclusions about the relationship between our cell therapy method and neuropathic pain.

To assess the safety in this phase I trial under the International Campaign for Cure of spinal cord Paralysis panel guidelines, we selected complete thoracic SCI individuals with no improvement

Table 4. Subjective changes of the research participants during the 2 years of the follow-up period.

Variable name	6 months before transplantation			At the time of transplantation			6 months after transplantation			12 months after transplantation			18 months after transplantation			24 months after transplantation		
Participant number	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Feeling of urination	–	–	–	=	=	=	=	=	=	=	+	+	=	=	=	=	=	=
Feeling of defecation	–	–	–	=	=	=	=	=	=	=	+	=	=	=	=	=	=	=
Urinary retention	–	–	–	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Urinary incontinence	+	+	+	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Constipation	–	+	–	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Fecal incontinence	+	+	+	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Trunk movements	+	+	+	=	=	=	↑	=	=	=	=	=	↑	=	=	=	=	=
Equilibrium in sitting position	+	–	+	=	=	=	=	=	=	=	+	=	↑	=	↑	=	=	=
Equilibrium in standing position	+	–	+	=	=	=	=	=	=	=	=	=	=	=	↑	=	+	↑

+: present at the time of evaluation.
–: absent at the time of evaluation.
=: unchanged compared to the previous evaluation.
↑: increased compared to the previous evaluation.

in the neurologic or functional scales after injury [27]. Regarding our determined criteria, only three volunteers were known eligible for enrollment in this pilot study, which is comparable with some of the previous phase I cell-based trials [7, 28]. In addition, we did not have randomly allocated control groups since it is not considered a necessary item for phase I clinical studies [29].

The cell injections were done between 23 and 75 months following SCI when all of the participants had stable neurologic conditions, and the possibility of any nonintervention-related recovery was almost zero [30]. One of our research participants had a significant response to this treatment so that her functional ability somewhat improved and her AIS changed from A (complete) to B (motor complete). Although rehabilitation could have been helpful in the neurological recovery of this participant, it cannot be regarded as the main cause of improvement because she had no change in her ISNCSCI and SCIM scores during the 6-month preoperative rehabilitation program. These results may offer a similar benefit for the OEC/MSC combination compared to the co-transplantation of Schwann cells and MSCs that led to an improvement in ISNCSCI scores and a change in the AIS in one of the participants [14]. Nevertheless, the small sample size of this safety-phase trial prevents us from commenting more on these findings.

The promising subjective results of this study were also similar to the reports of our previous Schwann cell/MSC combination therapies [11, 14]. These improvements, however, were associated with some degrees of progress in the self-care and motion scores of participant 2. Considering her old age and overweight, she had a remarkably lower initial SCIM score comparing to the other two participants. However, several months after the intervention, her SCIM score somewhat improved. This may reflect the improvement in this participant's neurological status and more emphasize the significance of the obtained favorable results. On the other hand, no progress was recorded in the total SCIM score of participant 3, and despite reporting urinary or defecation sensation, the bladder or bowel sphincter management scores were unchanged, which may be attributed to both severity and chronicity of the injury and the stable condition of the research participants.

Based on the obtained results and the numerous evidence on the outstanding regenerative properties of OECs and MSCs, the simultaneous application of these cells in human SCI seems justifiable. Owing to the use of the great regenerative potential of both cells, this combination can therefore be acknowledged as one of the most encouraging treatments of choice to overcome various obstacles in the functional restoration of the damaged spinal cord.

Overall, this pilot study proposes short-term safety for the concurrent administration of autologous mucosal OEC and bone marrow MSC into people with chronic, complete SCI. Based on the results of the assessed safety parameters and observing no serious adverse reaction in our three participants during at least 2 years of follow-up, this combination therapy is appeared to be a feasible and relatively safe treatment procedure for further studies. However, due to the various limitations of the present study such as its small number of participants, lacking control groups, and the inadequacy of objective measures, we cannot comment on the efficacy of this treatment approach with certainty. Therefore, it is required to design randomized controlled phase II trials with larger sample sizes and longer follow-up periods to further evaluate its safety and true efficacy in human SCI.

REFERENCES

1. Mehrabi S, Eftekhari S, Moradi F, Delaviz H, Pourheidar B, Azizi M, et al. Cell therapy in spinal cord injury: a mini-reivew. Basic Clin Neurosci. 2013;4:172–6.

2. Anna Z, Katarzyna J-W, Joanna C, Barczewska M, Joanna W, Wojciech M. Therapeutic potential of olfactory ensheathing cells and mesenchymal stem cells in spinal cord injuries. Stem Cells Int. 2017;2017:3978595.
3. Chou R-H, Lu C-Y, Wei-Lee, Fan J-R, Yu Y-L, Shyu W-C. The potential therapeutic applications of olfactory ensheathing cells in regenerative medicine. Cell Transplant. 2014;23:567–71.
4. Lankford KL, Sasaki M, Radtke C, Kocsis JD. Olfactory ensheathing cells exhibit unique migratory, phagocytic, and myelinating properties in the X-irradiated spinal cord not shared by Schwann cells. Glia. 2008;56:1664–78.
5. Gilmour AD, Reshamwala R, Wright AA, Ekberg JA, St John JA. Optimizing olfactory ensheathing cell transplantation for spinal cord injury repair. J Neurotrauma. 2020;37:817–29.
6. Taran R, Mamidi MK, Singh G, Dutta S, Parhar IS, John JP, et al. In vitro and in vivo neurogenic potential of mesenchymal stem cells isolated from different sources. J Biosci. 2014;39:157–69.
7. Tabakow P, Jarmundowicz W, Czapiga B, Fortuna W, Miedzybrodzki R, Czyz M, et al. Transplantation of autologous olfactory ensheathing cells in complete human spinal cord injury. Cell Transplant. 2013;22:1591–612.
8. Mendonça MVP, Larocca TF, de Freitas Souza BS, Villarreal CF, Silva LFM, Matos AC, et al. Safety and neurological assessments after autologous transplantation of bone marrow mesenchymal stem cells in subjects with chronic spinal cord injury. Stem Cell Res Ther. 2014;5:126.
9. Griffin JM, Bradke F. Therapeutic repair for spinal cord injury: combinatory approaches to address a multifaceted problem. EMBO Mol Med. 2020;12:e11505.
10. Zhang J, Chen H, Duan Z, Chen K, Liu Z, Zhang L, et al. The effects of co-transplantation of olfactory ensheathing cells and schwann cells on local inflammation environment in the contused spinal cord of rats. Mol Neurobiol. 2017;54:943–53.
11. Oraee-Yazdani S, Hafizi M, Zali A-R, Atashi A, Ashrafi F, Seddighi A-S, et al. Safety and possible outcome assessment of autologous Schwann cell and bone marrow mesenchymal stromal cell co-transplantation for treatment of patients with chronic spinal cord injury. Cytotherapy. 2013;15:782–91.
12. Chen L, Huang H, Xi H, Zhang F, Liu Y, Chen D, et al. A prospective randomized double-blind clinical trial using a combination of olfactory ensheathing cells and Schwann cells for the treatment of chronic complete spinal cord injuries. Cell Transplant. 2014;23 1_suppl:35–44.
13. Khuu HM, Patel N, Carter CS, Murray PR, Read EJ. Sterility testing of cell therapy products: parallel comparison of automated methods with a CFR-compliant method. Transfusion. 2006;46:2071–82.
14. Oraee-Yazdani S, Hafizi M, Atashi A, Ashrafi F, Seddighi A, Hashemi S, et al. Co-transplantation of autologous bone marrow mesenchymal stem cells and Schwann cells through cerebral spinal fluid for the treatment of patients with chronic spinal cord injury: safety and possible outcome. Spinal Cord. 2016;54:102–9.
15. Hosseini SM, Sani M, Haider KH, Dorvash M, Ziaee SM, Karimi A, et al. Concomitant use of mesenchymal stem cells and neural stem cells for treatment of spinal cord injury: a combo cell therapy approach. Neurosci Lett. 2018;668:138–46.
16. Cofano F, Boido M, Monticelli M, Zenga F, Ducati A, Vercelli A, et al. Mesenchymal stem cells for spinal cord injury: current options, limitations, and future of cell therapy. Int J Mol Sci. 2019;20:2698.
17. Deng Y, Liu Y, Zhu W, Bi X, Wang Y, Ye M, et al. The co-transplantation of human bone marrow stromal cells and embryo olfactory ensheathing cells as a new approach to treat spinal cord injury in a rat model. Cytotherapy. 2008;10:551–64.
18. Wu S, Cui G, Shao H, Du Z, Ng JC, Peng C. The cotransplantation of olfactory ensheathing cells with bone marrow mesenchymal stem cells exerts antiapoptotic effects in adult rats after spinal cord injury. Stem Cells Int. 2015;2015:516215.
19. Gomes ED, Mendes SS, Assunção-Silva RC, Teixeira FG, Pires AO, Anjo SI, et al. Co-transplantation of adipose tissue-derived stromal cells and olfactory ensheathing cells for spinal cord injury repair. Stem Cells. 2018;36:696–708.
20. Reshamwala R, Shah M, John JS, Ekberg J. The link between olfactory ensheathing cell survival and spinal cord injury repair: a commentary on common limitations of contemporary research. Neural Regen Res. 2020;15:1848–9.
21. Barnett SC, Chang L. Olfactory ensheathing cells and CNS repair: going solo or in need of a friend? Trends Neurosci. 2004;27:54–60.
22. Paul C, Samdani AF, Betz RR, Fischer I, Neuhuber B. Grafting of human bone marrow stromal cells into spinal cord injury: a comparison of delivery methods. Spine. 2009;34:328–34.
23. Satake K, Lou J, Lenke LG. Migration of mesenchymal stem cells through cerebrospinal fluid into injured spinal cord tissue. Spine. 2004;29:1971–9.
24. Li L, Adnan H, Xu B, Wang J, Wang C, Li F, et al. Effects of transplantation of olfactory ensheathing cells in chronic spinal cord injury: a systematic review and meta-analysis. Eur Spine J. 2015;24:919–30.
25. Macias MY, Syring MB, Pizzi MA, Crowe MJ, Alexanian AR, Kurpad SN. Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. Exp Neurol. 2006;201:335–48.
26. Lima C, Pratas-Vital J, Escada P, Hasse-Ferreira A, Capucho C, Peduzzi JD. Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. J Spinal Cord Med. 2006;29:191–203.
27. Tuszynski M, Steeves J, Fawcett J, Lammertse D, Kalichman M, Rask C, et al. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. Spinal Cord. 2007;45:222–31.
28. Feron F, Perry C, Cochrane J, Licina P, Nowitzke A, Urquhart S, et al. Autologous olfactory ensheathing cell transplantation in human spinal cord injury. Brain. 2005;128:2951–60.
29. Lammertse D, Tuszynski M, Steeves J, Curt A, Fawcett J, Rask C, et al. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: clinical trial design. Spinal Cord. 2007;45:232–42.
30. Fawcett J, Curt A, Steeves J, Coleman W, Tuszynski M, Lammertse D, et al. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. Spinal Cord. 2007;45:190–205.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr Mohammad Vasei and the staff of the cytogenetic department of Children's Medical Center.

AUTHOR CONTRIBUTIONS

All of the authors participated in drafting the article and revising it critically for important intellectual content. All authors read and approved the final manuscript.

FUNDING INFORMATION

This work was supported by the research grant from the Faculty of Medical Sciences, Tarbiat Modares University (Med-75939).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

This study was designed in accordance with the 1964 Declaration of Helsinki and was approved by the Ethics in Medical Research Committee of Tarbiat Modares University (IR.TMU.REC.1396.737). We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41393-021-00687-5.

Correspondence and requests for materials should be addressed to Mina Soufizomorrod, Saeed Oraee-Yazdani or Masoud Soleimani.