FULL-LENGTH ARTICLE

Clinical Research

Repeated subarachnoid administrations of allogeneic human umbilical cord mesenchymal stem cells for spinal cord injury: a phase 1/2 pilot study

Yang Yang^{1,†}, Mao Pang^{1,†}, Cong Du², Zhong-Yu Liu¹, Zi-Hao Chen¹, Nan-Xiang Wang¹, Liang-Ming Zhang¹, Yu-Yong Chen¹, Jian Mo¹, Jian-Wen Dong¹, Pei-Gen Xie¹, Qi-You Wang¹, Bin Liu^{1,*}, Li-Min Rong^{1,*}

¹ Department of Spine Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangdong Provincial Center for Engineering and Technology Research of Minimally Invasive Spine Surgery, Guangdong Provincial Center for Quality Control of Minimally Invasive Spine Surgery, Guangzhou, People's Republic of China

² Cell-Gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China

ARTICLE INFO

Article History:

Received 21 May 2020

Accepted 30 September 2020

Key Words:

clinical trial

efficacy

human umbilical cord mesenchymal stem cell

safety

spinal cord injury

subarachnoid transplantation

ABSTRACT

Background aims: Stem cell transplantation is a potential treatment for intractable spinal cord injury (SCI), and allogeneic human umbilical cord mesenchymal stem cells (hUC-MSCs) are a promising candidate because of the advantages of immune privilege, paracrine effect, immunomodulatory function, convenient collection procedure and little ethical concern, and there is an urgent need to develop a safe and effective protocol regarding their clinical application.

Methods: A prospective, single-center, single-arm study in which subjects received four subarachnoid transplantations of hUC-MSCs (1×10^6 cells/kg) monthly and were seen in follow-up four times (1, 3, 6 and 12 months after final administration) was conducted. At each scheduled time point, safety and efficacy indicators were collected and analyzed accordingly. Adverse events (AEs) were used as a safety indicator. American Spinal Injury Association (ASIA) and SCI Functional Rating Scale of the International Association of Neurorestoratology (IANR-SCIFRS) total scores at the fourth follow-up were determined as primary efficacy outcomes, whereas these two indicators at the remaining time points as well as scores of pinprick, light touch, motor and sphincter, muscle spasticity and spasm, autonomic system, bladder and bowel functions, residual urine volume (RUV) and magnetic resonance imaging (MRI) were secondary efficacy outcomes. Subgroup analysis of primary efficacy indicators was also performed.

Results: Safety and efficacy assessments were performed on 102 and 41 subjects, respectively. Mild AEs involving fever (14.1%), headache (4.2%), transient increase in muscle tension (1.6%) and dizziness (1.3%) were observed following hUC-MSC transplantation and resolved thoroughly after conservative treatments. There was no serious AE. ASIA and IANR-SCIFRS total scores revealed statistical increases when compared with the baselines at different time points during the study, mainly reflected in the improvement of pinprick, light touch, motor and sphincter scores. Moreover, subjects showed a continuous and remarkable decrease in muscle spasticity. Regarding muscle spasm, autonomic system, bladder and bowel functions, RUV and MRI, data/imaging at final follow-up showed significant improvements compared with those at first collection. Subgroup analysis found that hUC-MSC transplantation improved neurological functions regardless of injury characteristics, including level, severity and chronicity.

Conclusions: The authors' present protocol demonstrates that intrathecal administration of allogeneic hUC-MSCs at a dose of 10^6 cells/kg once a month for 4 months is safe and effective and leads to significant improvement in neurological dysfunction and recovery of quality of life.

© 2020 International Society for Cell & Gene Therapy. Published by Elsevier Inc. All rights reserved.

* Correspondence: Bin Liu, MD and Li-Min Rong, MD, Department of Spine Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangdong Provincial Center for Engineering and Technology Research of Minimally Invasive Spine Surgery, Guangdong Provincial Center for Quality Control of Minimally Invasive Spine Surgery, 600 Tianhe Rd, Guangzhou 510630, People's Republic of China.

E-mail addresses: liubin6@mail.sysu.edu.cn (B. Liu), ronglm@mail.sysu.edu.cn (L.-M. Rong).

† These authors contributed equally to this work.

Introduction

Spinal cord injury (SCI) is a devastating disease and often leads to lifelong disability, muscle spasm, sensory deficits, autonomic disturbances and bowel and bladder incontinence, all of which can cause tremendous difficulty for patients, but there has been a lack of any effective treatment up to now [1–3]. It is because of these sequelae that the quality of life for both patients and caregivers is significantly decreased. Thus, SCI is not only a severe health problem but also a great social burden [4,5]. To the best of the authors' knowledge, cell therapy seems to be a promising alternative for the treatment of SCI due to numerous advantages, including self-renewal, multi-lineage differentiation, inflammation control, immunomodulatory function, inhibition of cellular apoptosis, production of multiple trophic factors and remyelination of damaged axons [6–9]. However, cytotherapy is still in its infancy, and there are many disparities and uncertainties regarding subject selection, cellular type, transplantation timing, administration dose and delivery route in clinical trial protocols [7,8,10,11]. Hence, a standardized, well-designed clinical study is urgently required for the safe and effective treatment of SCI.

Among a variety of cell candidates for allogeneic transplantation, human umbilical cord mesenchymal stem cells (hUC-MSCs) are an attractive choice for treating intractable SCI because of their additional merits, including easy obtainment, rapid proliferation, lack of ethical controversy, immune privilege and paracrine effect [12,13]. Since the first subarachnoid administration of stem cells in a SCI subject in 2008, this convenient, minimally invasive method has been considered the safest and most effective approach for cell delivery because of less retention in other organs, reduced host immune response, more cell engraftment and better tissue sparing in the injured spinal cord [6–8,10,11,14]. Previous studies have suggested that transplanting a greater number of cells is advisable because of the fact that cell therapy has a dose-dependent effect. Thus, repeated cell administrations, which are facilitated by the ease of repeatability from intrathecal transplantation, seem to be more beneficial for SCI patients [7,8,10,15]. Here the authors present the results of a prospective phase 1/2 pilot trial that assesses the safety and efficacy of subarachnoid transplantation of four doses of allogeneic hUC-MSCs 10^6 cells/kg, with an interval of 1 month between each administration, in Chinese subjects with SCI (ClinicalTrials.gov identifier NCT02481440; Third Affiliated Hospital of Sun Yat-sen University Institutional Review Board academic approval nos. [2017]22 and [2017]24; Third Affiliated Hospital of Sun Yat-sen University Institutional Review Board ethical approval nos. [2018]02 and [2018]03).

Methods

The hUC-MSC medicament

The hUC-MSCs were prepared in the Good Manufacturing Practice Stem Cell Laboratory Facility of the Third Affiliated Hospital of Sun Yat-sen University. After obtaining informed consent forms from the parents of healthy full-term neonates, fresh umbilical cords were obtained immediately after birth and saved in sterile normal saline in a 4°C environment. Following the removal of arteries and vessels within umbilical cords, Wharton's jelly was split into pieces with volumes of less than 1 cm³; these tissues were then cultured on the bottom of plates. Approximately 1 week later, cell clones of hUC-MSCs could be observed around the Wharton's jelly slices. These primary hUC-MSCs were digested using TrypLE express (Gibco), and then single cells were adjusted to a concentration of 2×10^4 /cm² in each plate. The hUC-MSCs were cultured in medium consisting of minimum essential medium alpha (Gibco), GlutaMAX (Gibco) and PLUSTM MSC qualified cell culture supplement (Compass Biomedical) in a humidified

atmosphere at 37°C with 5% carbon dioxide. After reaching confluence above 90%, adherent spindle-shaped cells were passaged to continue proliferating (see supplementary Figure 1). Following a series of in-depth quality control tests to confirm conditions including but not limited to normal karyotype (number and morphology of chromosomes), cell viability (>95%), absence of microbial contamination (endotoxin, exotoxin, *Mycoplasma*, *Chlamydia*, bacteria, virus, fungus, *Treponema pallidum*), lack of tumorigenicity and tri-lineage differentiation potentials (osteogenesis, chondrogenesis, adipogenesis) *in vitro*, hUC-MSCs at passage five were detached for use in this trial. Before transplantation, phenotypic characterizations of hUC-MSCs were confirmed by flow cytometry, with positive rates of CD105, CD90, CD73 and CD44 no less than 95% and negative rates of CD45, CD34, CD11b and HLA-DR no more than 2% (see supplementary Figure 2).

For transportation, hUC-MSCs were suspended in 10 mL of sterile normal saline and maintained in a temperature-controlled environment of approximately 4°C under sterile and dark conditions. The stem cell medicament was double-packaged and transported as rapidly as possible to ensure infusion within 8 h after dissociation.

Study design

This was a prospective, single-center, single-arm study performed between March 2018 and March 2020. The study complied with the guidelines of the World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects as well as the principles of Good Clinical Practice.

Inclusion criteria of this clinical trial were as follows: (i) Chinese citizen (only in Chinese Mainland), (ii) complete or incomplete trauma-induced SCI (American Spinal Injury Association [ASIA] impairment scale classification A–D) that happened at least 2 months before recruitment (defined as chronic SCI in this study), (iii) aged between 18 and 65 years, (iv) agreed to participate in the study voluntarily and be followed up regularly for up to 12 months after the completion of hUC-MSC administration. Exclusion criteria were as follows: (i) intraspinal tumor, ankylosing spondylitis, myelitis or vascular abnormalities within the spinal cord parenchyma; (ii) severe comorbidities, including but not limited to craniocerebral injury, cutaneous back infection, psychiatric disease or cancer; (iii) pregnancy or lactation; (iv) predicted lifespan of less than 12 months following the end of hUC-MSC transplantation; (v) participation in any other stem cell-related clinical trials that might affect accurate neurological evaluations in the present trial; (vi) presence of coagulation diseases (prothrombin time >17.5 seconds, activated partial thromboplastin time >55 seconds or platelets < 100×10^9 /L); (vii) dysfunction of liver or kidneys (alanine transaminase >70 U/L, aspartate aminotransferase >80 U/L or serum creatinine >232 μ mol/L); (viii) inability to cooperatively complete follow-up post-transplantation; (ix) any medical condition that, in the opinion of investigators, may pose a safety risk to any subject in the study, confound safety or efficacy assessments or interfere with study participation. Rejection criteria were as follows: (i) misdiagnosis, (ii) use of any medication that may significantly impact the assessment accuracy of stem cell engraftment, (iii) absence of any evaluation outcome at any time point during the follow-up period, (iv) participation in any other stem cell-related clinical trials during the implementation of the study. Cessation criteria were as follows: (i) individual wishes of the subject; (ii) occurrence of any stem cell-associated serious adverse event (AE) that may aggravate neurological dysfunction, require prolongation of existing hospitalization or hospital readmission, impair consciousness, be life-threatening or even lead to death in any subject; (iii) detection of any major mistake in the present protocol during the implementation of the clinical trial; (iv) national administration agency requirement that the clinical trial be halted.

Figure 1. Flow chart of the study. (Color version of figure is available online).

Study protocol

Informed consent forms that had the approval of the institutional review board were obtained from all participants before recruitment. In this clinical study, subarachnoid transplantation of hUC-MSCs was performed a total of four times per subject, with the delivery dose of 1×10^6 cells/kg based on results from the authors' pre-clinical studies (manuscripts in revision). For the first administration of stem cells, maximum lumbar flexion of the subject was maintained, and cerebrospinal fluid release with a volume of approximately 10 mL was performed. After gentle swirling and tapping, hUC-MSC suspension with a volume of 10 mL was administered manually at the L4–L5 level as slowly as possible to minimize the possibility of any complication associated with intrathecal transplantation, including headache, dizziness, nausea or vomiting. This was followed by a 2-min delay before the injection needle was withdrawn, ensuring that all stem cells were grafted within the dural sac. An immunosuppressant was not given to any subject because of the low immunogenicity of hUC-MSCs [16]. One month later, each subject underwent a second subarachnoid engraftment of hUC-MSCs. Following this pattern, each subject received a third and fourth stem cell transplantation.

During the intervention period of 4 months, all subjects received standardized care. After the completion of cytotherapy, each subject was regularly followed up in the hospital at four time points, determined at 1, 3, 6 and 12 months following the final administration of hUC-MSCs. At each time point of administration (first, second, third and fourth transplantation) and follow-up (first, second, third and fourth follow-up), safety and efficacy indicators were collected by two independent assessors. An AE referred to any unwanted symptom or sign in a clinical investigation subject and did not necessarily have a causal relationship with the applied intervention [17]. During the intervention and follow-up periods of this trial, any AE was identified rapidly and managed properly. Collected AEs were then classified according to the Medical Dictionary for Regulatory Activities v18.1, and the maximum intensity and relationship of any AE with hUC-MSC administration were identified. Efficacy indicators included ASIA score and the SCI Functional Rating Scale of the International Association of Neurorestoratology (IANR-SCIFRS) for the recovery of

Table 1

Scores on the ASIA scale.

Scale	Time point	Mean $\pm$ SD	Median (IQR)	P value
ASIA total score	1st Tr	158.15 $\pm$ 70.93		–
	2nd Tr	163.80 $\pm$ 72.53		0.002
	3rd Tr	168.41 $\pm$ 74.16		< 0.001
	4th Tr	172.00 $\pm$ 73.51		< 0.001
	1st FU	176.05 $\pm$ 71.88		< 0.001
	2nd FU	178.39 $\pm$ 71.51		< 0.001
	3rd FU	182.44 $\pm$ 71.00		< 0.001
	4th FU	183.88 $\pm$ 69.76		< 0.001
Pinprick score	1st Tr		60.00 (29.00–74.00)	–
	2nd Tr		63.00 (31.50–74.50)	< 0.001
	3rd Tr		68.00 (34.00–77.00)	< 0.001
	4th Tr		66.00 (35.00–77.00)	< 0.001
	1st FU		68.00 (38.00–78.00)	< 0.001
	2nd FU		70.00 (39.50–78.00)	< 0.001
	3rd FU		74.00 (44.00–79.00)	< 0.001
	4th FU		72.00 (44.50–81.50)	< 0.001
Light touch score	1st Tr		63.00 (30.00–76.00)	–
	2nd Tr		64.00 (35.00–76.50)	< 0.001
	3rd Tr		68.00 (35.00–78.00)	< 0.001
	4th Tr		66.00 (41.00–80.00)	< 0.001
	1st FU		73.00 (42.00–81.50)	< 0.001
	2nd FU		74.00 (43.00–81.50)	< 0.001
	3rd FU		74.00 (49.50–82.50)	< 0.001
	4th FU		74.00 (50.00–84.50)	< 0.001
Motor score	1st Tr	44.44 $\pm$ 20.87		–
	2nd Tr	45.39 $\pm$ 21.22		0.079
	3rd Tr	45.76 $\pm$ 21.31		0.029
	4th Tr	45.98 $\pm$ 21.14		0.027
	1st FU	46.71 $\pm$ 21.65		0.005
	2nd FU	46.73 $\pm$ 21.20		0.006
	3rd FU	47.34 $\pm$ 21.17		0.003
	4th FU	47.90 $\pm$ 21.02		0.003

Significant *P* values are highlighted in bold. Paired samples *t*-test or Wilcoxon signed-rank test was applied when compared with the first transplantation at each time point after Bonferroni correction.

FU, follow-up; IQR, interquartile range; SD, standard deviation; Tr, transplantation.

spinal cord function [18,19], the international standards to document remaining autonomic function after spinal cord injury [20], Penn and modified Ashworth scales for the evaluation of spasms and spasticity of affected muscles [21,22], Geffner scale for the study of bladder function and Neurogenic Bowel Dysfunction score [23,24], residual urine volume (RUV) and magnetic resonance imaging. In this trial, ASIA and IANR-SCIFRS total scores at the fourth follow-up were determined as primary outcomes, whereas these two indicators at the remaining time points and the other efficacy indicators were secondary outcomes.

Sample size evaluation

A difference test was used to predict sample size in this study. The two-tailed significance level (α) was 0.05. To provide 80% power ($1-\beta$) to detect any differences in changes in primary outcomes, β was set at 0.2. Based on the results of the authors' preliminary clinical trial, the standard deviation (σ) in ASIA and IANR-SCIFRS total scores was 56 and 10, respectively. According to expert consensus from the spine surgery, rehabilitation and neurology departments, minimum improvements of 25 in the ASIA total score and 5 in the IANR-SCIFRS total score were believed to have clinical significance; therefore, ε was set at 25 and 5, respectively. Hence, a minimum of 40 finally included subjects was sufficient to detect differences between pre- and post-cytotherapy.

Statistical analysis

In this clinical study, three analysis sets were established. For the safety analysis set, all AEs were obtained from those who had

received at least one dose of hUC-MSCs. In the full analysis set, subjects were excluded if they received no intervention or never gave any evaluation outcome. For the per protocol set, subjects should achieve at least 90% integrity for all the following criteria: (i) underwent a total of four times of hUC-MSCs administration, (ii) showed no violation of the present protocol, (iii) completed the full follow-up, (iv) obtained all required data. Continuous variables were expressed as means $\pm$ standard deviations or median with interquartile range according to normality, whereas categorical data were given as frequencies. In terms of efficacy indicators collected at multiple (≥ 3) time points, analysis of variance for repeated measurement or Friedman test was used to detect overall statistical significance of normal or non-normal continuous data. Following the Bonferroni correction, the paired samples *t*-test and Wilcoxon signed-rank test were applied to analyze normal and non-normal continuous variables, respectively. According to injury characteristics, including level, severity and chronicity, subgroup analysis of two primary efficacy indicators (ASIA and IANR-SCIFRS total scores at the fourth follow-up) was performed using paired samples *t*-test. SPSS Statistics 22.0 was used to perform all statistical analyses, and $P < 0.05$ was considered statistically significant. All graphs were made with Prism 5.04 (GraphPad Software Inc) for Windows. A flow chart of subject enrollment, intervention, follow-up and analysis is presented in Figure 1.

Results

For both the safety analysis set and the full analysis set, 102 subjects were included. A total of 383 intrathecal transplantations of hUC-MSCs were performed. During the cytotherapy, observed AEs were as follows: 54 episodes of fever (14.1%), 16 episodes of

Figure 2. Scores in ASIA scale. (A) ASIA total score with normal distribution presented in histogram. (B) Median of ASIA light touch score with non-normal distribution presented in line chart. (C) ASIA motor score with normal distribution presented in histogram. (D) Median of ASIA pinprick score with non-normal distribution presented in line chart. NS = $P > 0.05$ when compared with the first transplantation, * $P < 0.05$ when compared with the first transplantation, ** $P < 0.001$ when compared with the first transplantation. ASIA, American Spinal Injury Association; FU, follow-up; NS, no significance; Tr, transplantation. (Color version of figure is available online).

headache (4.2%), six episodes of transient increase in muscle tension (1.6%) and five episodes of dizziness (1.3%). Most of these AEs were of mild intensity and resolved thoroughly after medication, physiotherapy and bed rest. No severe AE that conditioned the withdrawal of a subject from the present study was observed.

For the per protocol set that was used to assess efficacy of stem cell therapy, 41 subjects were included (male:female = 33:8) (see supplementary Table 1). Participant ages ranged from 18 to 65 years, with a mean value of 38.1 ± 11.0 years. According to the causes given for SCI, 18 subjects were injured in traffic accidents and 14 subjects were injured after a fall from height. Spinal cord function in the remaining nine subjects was damaged by other kinds of violence. With respect to the injury level of SCI, 24 subjects (58.5%) had a lesion at the cervical level (C1–C7), seven subjects (17.1%) at the thoracic level (T1–T9) and the remaining 10 subjects (24.4%) at the thoracolumbar level (T10–L1). In terms of lesion severity, there were 13 subjects (31.7%) suffering complete injury and 28 subjects (68.3%) with incomplete damage. Regarding the chronicity of SCI, the time from occurrence of injury to initiation of first stem cell therapy was 2 months to 240 months, with a mean value of 45.15 ± 59.78 months.

With respect to efficacy outcomes collected at multiple time points, analysis of variance for repeated measurement and Friedman test found that all these indicators showed overall statistical significance. In terms of ASIA total score, pinprick score and light touch score, the data collected at the remaining seven time points showed statistical improvements when compared with those at the first transplantation (Table 1, Figure 2). Compared with the baselines of motor score, IANR-SCIFRS total score and sphincter score, subjects' data started to reveal significant increases at the first follow-up, third transplantation and fourth transplantation. With regard to the modified Ashworth scale, remarkable decreases could be observed at the first, second and fourth follow-ups when compared with the score at

the first transplantation. Regarding the international standards to document remaining autonomic function after spinal cord injury, Penn scale, Geffner scale, Neurogenic Bowel Dysfunction score, RUV and magnetic resonance imaging, subjects' data and imaging at final follow-up showed significant improvements compared with those at initial collection (Tables 1, 2; Figures 2, 3; also see supplementary Figure 3). With respect to subgroup analysis of ASIA and IANR-SCIFRS total scores at the fourth follow-up, subjects' scores revealed significant increases in all subgroups when compared with those at the first transplantation, categorized by lesion level (cervical, thoracic, thoracolumbar), damage severity (complete, incomplete) and injury chronicity (early chronic phase [2–12 months], late chronic phase [more than 12 months]) (Table 3).

Discussion

In this clinical study, the hUC-MSc medicament was well prepared before administration to subjects with chronic SCI. Specifically, transplanted stem cells were cultured in medium free of any animal-derived supplement because there is growing concern over transmission of xenogenic infections or transfer of xenoantigens in clinical medicaments. It is estimated that a maximum of 30 mg bovine protein can be transferred during cultivation of 10^8 MSCs if they are cultured in medium with fetal bovine or calf serum [25,26]. Thus, the repeated administrations in this trial that were needed to deliver multiple doses benefited more from the advantage of a medium lacking xenogenic protein. Moreover, various quality control measures and rigorous transportation requirements were necessary in the authors' study because a well-characterized stem cell population is a critical component of safety in clinical application [27].

Regarding the safety of hUC-MSc delivery, the majority of subjects showed no AEs. Among the remaining subjects with different kinds of

Table 2
Scores on IANR-SCIFRS, Ashworth, ISAFSCI, Penn, Geffner and NBD scales and RUV.

Scale	Time point	Mean $\pm$ SD	Median (IQR)	P value
IANR-SCIFRS total score	1st Tr	24.54 $\pm$ 9.82		–
	2nd Tr	25.71 $\pm$ 10.32		0.027
	3rd Tr	26.05 $\pm$ 10.15		0.001
	4th Tr	26.85 $\pm$ 10.36		< 0.001
	1st FU	27.56 $\pm$ 10.03		< 0.001
	2nd FU	28.44 $\pm$ 10.28		< 0.001
	3rd FU	28.93 $\pm$ 10.26		< 0.001
	4th FU	29.49 $\pm$ 10.47		< 0.001
Sphincter score	1st Tr		1.00 (0.00–2.50)	–
	2nd Tr		1.00 (0.00–3.00)	0.025
	3rd Tr		2.00 (0.00–3.00)	0.008
	4th Tr		2.00 (0.00–3.00)	0.002
	1st FU		2.00 (1.00–3.00)	< 0.001
	2nd FU		2.00 (1.00–3.00)	< 0.001
	3rd FU		2.00 (1.00–3.00)	< 0.001
	4th FU		2.00 (1.00–4.00)	< 0.001
Ashworth score	1st Tr		3.00 (0.00–6.00)	–
	1st FU		2.50 (0.00–4.50)	0.003
	2nd FU		2.00 (0.00–4.00)	0.001
	4th FU		2.00 (0.00–4.00)	0.001
ISAFSCI score	1st Tr		11.00 (10.00–12.00)	–
	4th FU		12.00 (11.00–14.00)	< 0.001
Penn score	1st Tr		1.00 (0.00–3.00)	–
	4th FU		1.00 (0.00–2.00)	0.009
Geffner score	1st Tr		2.00 (0.00–4.00)	–
	4th FU		3.00 (2.00–4.00)	0.001
NBD score	1st Tr		6.00 (2.00–12.50)	–
	4th FU		3.00 (2.00–10.00)	0.005
RUV	1st Tr		67.65 (31.58–144.50)	–
	4th FU		27.90 (8.83–88.50)	< 0.001

Significant P values are highlighted in bold. Paired samples t-test or Wilcoxon signed-rank test was applied when compared with the first transplantation at each time point after Bonferroni correction.

FU, follow-up; IQR, interquartile range; ISAFSCI, international standards to document remaining autonomic function after spinal cord injury; NBD, Neurogenic Bowel Dysfunction; SD, standard deviation; Tr, transplantation.

Figure 3. Scores in IANR-SCIFRS and NBD. (A) IANR-SCIFRS total score with normal distribution presented in histogram. (B) Classified evaluation of the total functional scores in 41 included subjects according to the IANR-SCIFRS scale. In this scale, a global score that ranges between 34 and 47 represents a slight handicap, a global score that ranges between 17 and 33 represents a medium handicap and a global score that ranges between 0 and 16 represents a severe handicap. (C) Median of IANR-SCIFRS sphincter score with non-normal distribution presented in line chart. (D) Classified evaluation of bowel functional scores in 41 included subjects according to the NBD scale. In this scale, a global score that ranges between 0 and 6 represents a very minor dysfunction, a global score that ranges between 7 and 9 represents a minor dysfunction, a global score that ranges between 10 and 13 represents a moderate dysfunction and a global score that is no less than 14 represents a severe dysfunction. NS = $P > 0.05$ when compared with the first transplantation. * $P < 0.05$ when compared with the first transplantation, ** $P < 0.001$ when compared with the first transplantation. NBD, Neurogenic Bowel Dysfunction; NS, no significance; Tr, transplantation; FU, follow-up; IANR-SCIFRS, SCI Functional Rating Scale of the International Association of Neurorestoratology. (Color version of figure is available online).

AEs, fever was most likely to be observed. Consistent with one report [28], fever commonly occurred within 24 h after hUC-MSC transplantation and could be considered a mild immunoreaction during cytotherapy. All fevers in this study were transient, with a maximum temperature below 40.0°C and most fevers no more than 38.8°C. After physical cooling and medication, fever mostly resolved within 24 h. Moreover, other kinds of AEs, including headache, dizziness and transient increase in muscle tension, showed low incidences (less than 5%) and were relieved thoroughly following conservative treatments. The possible cause of these AEs is inflammation response triggered by hUC-MSC administration. In general, subarachnoid transplantation of allogeneic hUC-MSCs does not expose subjects to a dangerous condition.

With respect to the efficacy of cytotherapy, it is necessary to know the selection of subjects who may receive benefits from this novel treatment before implementation because injury characteristics, including severity and chronicity, are found to influence clinical outcomes of stem cell therapy for SCI [15,29]. A careful evaluation of the morphology of the SCI site prior to subject enrollment is a prerequisite, and efficacy of cytotherapy should not be expected if there is no acceptable continuity of damaged spinal cord [8,29]. All subjects enrolled in this trial were suffering long-standing and stable neurological dysfunction, and those subjects with SCI in the acute phase were excluded. This is because transplantation at the chronic stage, as compared with the acute period, may provide a more favorable and less inflammatory environment for stem cell survival and engraftment [30]. This is especially beneficial for SCI repair because

the survival of transplanted stem cells, as much as possible, is required to trigger neurological recovery [31]. Moreover, following the occurrence of SCI, some subjects possibly show different levels of spontaneous repair of neurological function during the first few days, and spinal cord function tends to be mostly stable at 2 months after primary injury [32]. Thus, for the purpose of accurate assessment of the therapeutic efficacy of hUC-MSCs, the authors only recruited subjects whose chronicity of SCI was no less than 2 months. In the authors' study, it is possibly due to the great severity of injury that some subjects showed no significant recovery of neurological function after hUC-MSC administration.

In this trial, subjects had SCI at the cervical, thoracic or thoracolumbar level. Neurological recovery could be observed in these subjects after intrathecal administration of hUC-MSCs in the lumbar region. This is possibly because stem cells travel through cerebrospinal fluid and accumulate at the injury site in the spinal cord as a result of chemotactic "homing" signaling and interaction with adhesion molecules [33]. Regarding the optimal transplantation dose and administration interval of stem cells, clinical experience in SCI therapy is limited nowadays, and detailed criteria have not been fully established. It is suggested that the dosage for each administration vary from 10^6 to 10^9 cells and the transplantation interval range from a few days to several months [15]. Based on these recommendations, hUC-MSC transplantation with four doses of 10^6 cells/kg and an interval of 1 month between each administration was initiated in this study. It is also hypothesized that using multiple administrations increases the number of stem cells that engraft at the injury site so

Table 3
Subgroup analysis of ASIA and IANR-SCIFRS total scores.

Subgroup	Time point	Mean $\pm$ SD	P value
ASIA total score			
Cervical level	1st Tr	125.96 $\pm$ 71.31	–
	4th FU	156.50 $\pm$ 74.11	< 0.001
Thoracic level	1st Tr	180.57 $\pm$ 22.29	–
	4th FU	203.57 $\pm$ 29.64	0.032
Thoracolumbar level	1st Tr	219.70 $\pm$ 40.88	–
	4th FU	235.80 $\pm$ 41.33	0.003
Complete injury	1st Tr	125.38 $\pm$ 61.10	–
	4th FU	145.31 $\pm$ 60.95	0.005
Incomplete injury	1st Tr	173.36 $\pm$ 70.98	–
	4th FU	201.71 $\pm$ 67.17	< 0.001
Early chronic phase	1st Tr	150.35 $\pm$ 60.41	–
	4th FU	176.47 $\pm$ 65.17	0.001
Late chronic phase	1st Tr	163.67 $\pm$ 78.32	–
	4th FU	189.13 $\pm$ 73.75	< 0.001
IANR-SCIFRS total score			
Cervical level	1st Tr	19.79 $\pm$ 9.29	–
	4th FU	24.75 $\pm$ 10.29	< 0.001
Thoracic level	1st Tr	28.14 $\pm$ 6.20	–
	4th FU	33.29 $\pm$ 6.29	0.029
Thoracolumbar level	1st Tr	33.40 $\pm$ 5.02	–
	4th FU	38.20 $\pm$ 5.88	< 0.001
Complete injury	1st Tr	22.77 $\pm$ 9.11	–
	4th FU	26.62 $\pm$ 9.93	0.001
Incomplete injury	1st Tr	25.36 $\pm$ 10.19	–
	4th FU	30.82 $\pm$ 10.62	< 0.001
Early chronic phase	1st Tr	23.29 $\pm$ 7.54	–
	4th FU	28.71 $\pm$ 9.26	0.003
Late chronic phase	1st Tr	25.42 $\pm$ 11.24	–
	4th FU	30.04 $\pm$ 11.41	< 0.001

Significant *P* values are highlighted in bold. Paired samples *t*-test was applied when comparing between the first transplantation and the fourth follow-up.

FU, follow-up; SD, standard deviation; Tr, transplantation.

that over time a graft size comparable to that of a directly injected graft can be obtained, benefiting reconstruction of neurological structures [33]. However, for subjects with different injury characteristics, the optimal protocol for hUC-MSC transplantation, including infusion dose, delivery times, administration interval and transplantation approach, has yet to be explored.

In the authors' study, the two primary outcomes—IANR-SCIFRS and ASIA total scores at the final follow-up—showed remarkable improvements when compared with baseline data, indicating the therapeutic efficacy of hUC-MSCs in treating SCI. Subgroup analysis also demonstrated that stem cell therapy could improve neurological dysfunction regardless of injury characteristics, including lesions at the cervical, thoracic and thoracolumbar levels; complete and incomplete damage; and early and late chronic phases. Perhaps the aforementioned positive results are due to sufficient quantity of transplanted stem cells and long observation period. In addition, in different aspects of neurological disability caused by chronic paraplegia, sensation was found to recover more quickly and significantly than motion and sphincter after intrathecal transplantation of hUC-MSCs. Moreover, the secondary outcomes, including muscle spasm, autonomic system and bladder and bowel functions, improved remarkably, supporting the application of stem cell therapy in clinic. Regarding other secondary efficacy indicators, early and progressive improvement of muscle spasticity is a beneficial outcome of cytotherapy [7,8,31], and a decrease in RUV can be observed prior to restoration of other neurological functions [11]. Similar to these reports, both primary and secondary efficacy outcomes in the authors' study are in favor of clinical application of stem cell therapy. Furthermore, most subjects in this trial experienced progressive improvement in neurological functions without reaching a plateau at the end of follow-up. With respect to therapeutic mechanisms of hUC-MSCs, a paracrine effect through released beneficial cytokines, which

promote host axonal growth and rejuvenate preserved but non-functional circuits, rather than a mechanism of nerve pathway regeneration, seems to be more reasonable [7,8,31]. Another positive function is eliminating glial scars within damaged spinal cord and thus benefiting regeneration of remyelinated axons [34]. Moreover, transplantation of hUC-MSCs can inhibit the polarization of M1 microglia/macrophages while promoting M2 polarization by reducing the inflammatory response at the injured site [35–37]. However, detailed cellular and molecular mechanisms regarding hUC-MSC therapy are not clear, and thus in-depth investigations are strongly needed. Such studies would be especially beneficial for increasing the therapeutic efficacy of hUC-MSCs. For example, the separation and transplantation of functional subpopulations of hUC-MSCs may accelerate neurological recovery more significantly [38].

In this trial, it was observed that two subjects showed partial retreat of sensation recovery at the third or fourth follow-up, although the overall improvement in sensation was not affected. Moreover, after receiving hUC-MSCs administration, one subject regained muscle spasticity although transient decrease of modified Ashworth scale could be found, albeit showing no effect to the final conclusion in this study. The reasons for these phenomena are not clear and need to be investigated in detail.

In summary, the strengths of this prospective trial are recruitment of the largest sample size so far, inclusion of autonomic system function assessment, enrollment of Asian subjects and accurate evaluation of neurological function recovery by measuring some efficacy indicators as many as eight times. This phase 1/2 pilot study would contribute to the initiation of a prospective, multicenter, randomized, placebo-controlled clinical trial in the future. However, this work is not devoid of intrinsic shortcomings. First, it is not a randomized controlled study, and thus potential inclusion bias of subjects might have influenced the assessment accuracy. Moreover, acquisition of some efficacy indicators was probably affected by subjects' mental status, leading to some biases in outcomes. Hence, high-quality studies with better design and larger sample size are needed to further justify the authors' findings.

Conclusions

In the absence of the best treatment guidelines for patients suffering from SCI, especially for the Asian population, the authors' present protocol demonstrates that intrathecal administration of allogeneic hUC-MSCs at a dose of 10^6 cells/kg once a month for 4 months is safe and effective and leads to significant improvement in neurological dysfunction and recovery of quality of life.

Funding

This work was supported by grants from the National Key Research and Development Program of China (2017YFA0105400), the Key Research and Development Program of Guangdong Province (20180236), the Clinical Innovation Research Program of Guangzhou Regenerative Medicine and Health Guangdong Laboratory (2018GZR0201006), the National Natural Science Foundation of China (31470949, 81772349), the Guangzhou Science and Technology Project (201704020221, 201704020049, 201707010115), the Guangdong Basic and Applied Basic Research Foundation (2019A1515110814, 2020A1515010171) and the Medical Scientific Research Foundation of Guangdong Province (A2018547).

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

Conception and design of the study: Li-Min Rong, Bin Liu, Yang Yang, Mao Pang. Acquisition of data: Cong Du, Zhong-Yu Liu, Zi-Hao Chen, Nan-Xiang Wang, Liang-Ming Zhang. Analysis and interpretation of data: Yu-Yong Chen, Jian Mo, Jian-Wen Dong, Pei-Gen Xie, Qi-You Wang. Drafting or revising the manuscript: Yang Yang, Mao Pang. All authors have approved the final article.

Acknowledgments

The authors thank Lian-Xiong Yuan for performing the statistical analysis for this study.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.jcyt.2020.09.012](https://doi.org/10.1016/j.jcyt.2020.09.012).

References

- [1] Wagner FB, Mignardot JB, Le Goff-Mignardot CG, Demesmaeker R, Komi S, Capogrosso M, et al. Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature* 2018;563(7729):65–71.
- [2] Silvestro S, Bramanti P, Trubiani O, Mazzon E. Stem Cells Therapy for Spinal Cord Injury: An Overview of Clinical Trials. *Int J Mol Sci* 2020;21(2):E659.
- [3] Ahuja CS, Nori S, Tetreault L, Wilson J, Kwon B, Harrop J, et al. Traumatic Spinal Cord Injury—Repair and Regeneration. *Neurosurgery* 2017;80(3S):S9–22.
- [4] Boswell-Ruys CL, Lewis CRH, Wijesuriya NS, McBain RA, Lee BB, McKenzie DK, et al. Impact of respiratory muscle training on respiratory muscle strength, respiratory function and quality of life in individuals with tetraplegia: a randomised clinical trial. *Thorax* 2020;75(3):279–88.
- [5] McKay RC, Wuerstl KR, Casemore S, Clarke TY, McBride CB, Gainforth HL, et al. Guidance for behavioural interventions aiming to support family support providers of people with spinal cord injury: a scoping review. *Soc Sci Med* 2020;246:112456.
- [6] Satti HS, Waheed A, Ahmed P, Ahmed K, Akram Z, Aziz T, et al. Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study. *Cytotherapy* 2016;18(4):518–22.
- [7] Vaquero J, Zurita M, Rico MA, Bonilla C, Aguayo C, Montilla J, et al. An approach to personalized cell therapy in chronic complete paraplegia: The Puerta de Hierro phase I/II clinical trial. *Cytotherapy* 2016;18(8):1025–36.
- [8] Vaquero J, Zurita M, Rico MA, Bonilla C, Aguayo C, Fernández C, et al. Repeated subarachnoid administrations of autologous mesenchymal stromal cells supported in autologous plasma improve quality of life in patients suffering incomplete spinal cord injury. *Cytotherapy* 2017;19(3):349–59.
- [9] Shende P, Subedi M. Pathophysiology, mechanisms and applications of mesenchymal stem cells for the treatment of spinal cord injury. *Biomed Pharmacother* 2017;91:693–706.
- [10] Vaquero J, Zurita M, Rico MA, Aguayo C, Bonilla C, Marin E, et al. Intrathecal administration of autologous mesenchymal stromal cells for spinal cord injury: safety and efficacy of the 100/3 guideline. *Cytotherapy* 2018;20(6):806–19.
- [11] Vaquero J, Zurita M, Rico MA, Aguayo C, Fernandez C, Rodriguez-Boto G, et al. Cell therapy with autologous mesenchymal stromal cells in post-traumatic syringomyelia. *Cytotherapy* 2018;20(6):796–805.
- [12] Sun L, Wang F, Chen H, Liu D, Qu T, Li X, et al. Co-Transplantation of Human Umbilical Cord Mesenchymal Stem Cells and Human Neural Stem Cells Improves the Outcome in Rats with Spinal Cord Injury. *Cell Transplant* 2019;28(7):893–906.
- [13] Peng C, Li Y, Lu L, Zhu J, Li H, Hu J. Efficient One-Step Induction of Human Umbilical Cord-Derived Mesenchymal Stem Cells (UC-MSCs) Produces MSC-Derived Neurospheres (MSC-NS) with Unique Transcriptional Profile and Enhanced Neurogenic and Angiogenic Secretomes. *Stem Cells Int* 2019;2019:9208173.
- [14] Krupa P, Vackova I, Ruzicka J, Zaviscka K, Dubisova J, Koci Z, et al. The Effect of Human Mesenchymal Stem Cells Derived from Wharton's Jelly in Spinal Cord Injury Treatment Is Dose-Dependent and Can Be Facilitated by Repeated Application. *Int J Mol Sci* 2018;19(5):E1503.
- [15] Fawcett JW, Curt A, Steeves JD, Coleman WP, Tuszynski MH, 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(3):190–205.
- [16] Li Shengying, Wang Yuxia, Guan Liping, Ji Mingli. Characteristics of Human Umbilical Cord Mesenchymal Stem Cells During Ex Vivo Expansion. *Mol Med Rep* 2015;12(3):4320–5.
- [17] Koda M, Hanaoka H, Sato T, Fujii Y, Hanawa M, Takahashi S, et al. Study protocol for the G-SPRIT trial: a randomised, placebo-controlled, double-blinded phase III trial of granulocyte colony-stimulating factor-mediated neuroprotection for acute spinal cord injury. *BMJ Open* 2018;8(5):e019083.
- [18] Burns S, Biering-Sørensen F, Donovan W, Graves DE, Jha A, Johansen M, et al. International standards for neurological classification of spinal cord injury, revised 2011. *Top Spinal Cord Inj Rehabil* 2012;18(1):85–99.
- [19] Derakhshanrad N, Saberi H, Yekaninejad MS, Joghataei MT. Subcutaneous granulocyte colony-stimulating factor administration for subacute traumatic spinal cord injuries, report of neurological and functional outcomes: a double-blind randomized controlled clinical trial. *J Neurosurg Spine* 2018;30(1):19–30.
- [20] Round AM, Park SE, Walden K, Noonan VK, Townson AF, Krassioukov AV. An evaluation of the International Standards to Document Remaining Autonomic Function after Spinal Cord Injury: input from the international community. *Spinal Cord* 2017;55(2):198–203.
- [21] Penn RD, Savoy SM, Corcos D, Latash M, Gottlieb G, Parke B, et al. Intrathecal baclofen for severe spinal spasticity. *N Engl J Med* 1989;320(23):1517–21.
- [22] Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987;67(2):206–7.
- [23] Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, Aua AH, et al. Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. *Cell Transplant* 2008;17(12):1277–93.
- [24] Krogh K, Christensen P, Sabroe S, Laurberg S. Neurogenic bowel dysfunction score. *Spinal Cord* 2006;44(10):625–31.
- [25] Spees JL, Gregory CA, Singh H, Tucker HA, Peister A, Lynch PJ, et al. Internalized antigens must be removed to prepare hypoinnate mesenchymal stem cells for cell and gene therapy. *Mol Ther* 2004;9(5):747–56.
- [26] Joswig AJ, Mitchell A, Cummings KJ, Levine GJ, Gregory CA, 3rd Smith R, et al. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. *Stem Cell Res Ther* 2017;8(1):42.
- [27] Anderson KD, Guest JD, Dietrich WD, Bartlett Bunge M, Curiel R, Dididze M, et al. Safety of Autologous Human Schwann Cell Transplantation in Subacute Thoracic Spinal Cord Injury. *J Neurotrauma* 2017;34(21):2950–63.
- [28] Dai G, Liu X, Zhang Z, Yang Z, Dai Y, Xu R. Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. *Brain Res* 2013;1533:73–9.
- [29] Vaquero J, Zurita M. Cell transplantation in paraplegic patients: the importance of properly assessing the spinal cord morphology. *Clin Transplant* 2013;27(6):968–71.
- [30] Levi AD, Anderson KD, Okonkwo DO, Park P, Bryce TN, Kurpad SN, et al. Clinical Outcomes from a Multi-Center Study of Human Neural Stem Cell Transplantation in Chronic Cervical Spinal Cord Injury. *J Neurotrauma* 2019;36(6):891–902.
- [31] Shin JC, Kim KN, Yoo J, Kim IS, Yun S, Lee H, et al. Clinical Trial of Human Fetal Brain-Derived Neural Stem/Progenitor Cell Transplantation in Patients with Traumatic Cervical Spinal Cord Injury. *Neural Plast* 2015;2015:630932.
- [32] Hur JW, Cho TH, Park DH, Lee JB, Park JY, Chung YG. Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: a human trial. *J Spinal Cord Med* 2016;39(6):655–64.
- [33] Bansal H, Verma P, Agrawal A, Leon J, Sundell IB, Koka PS. Autologous Bone Marrow-Derived Stem Cells in Spinal Cord Injury. *J Stem Cells* 2016;11(1):51–61.
- [34] Park JH, Kim DY, Sung IY, Choi GH, Jeon MH, Kim KK, et al. Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. *Neurosurgery* 2012;70(5):1238–47.
- [35] Chen C, Chen F, Yao C, Shu S, Feng J, Hu X, et al. Intrathecal Injection of Human Umbilical Cord-Derived Mesenchymal Stem Cells Ameliorates Neuropathic Pain in Rats. *Neurochem Res* 2016;41(12):3250–60.
- [36] Bao CS, Li XL, Liu L, Wang B, Yang FB, Chen LG. Transplantation of human umbilical cord mesenchymal stem cells promotes functional recovery after spinal cord injury by blocking the expression of IL-7. *Eur Rev Med Pharmacol Sci* 2018;22(19):6436–47.
- [37] Wu LL, Pan XM, Chen HH, Fu XY, Jiang J, Ding MX. Repairing and Analgesic Effects of Umbilical Cord Mesenchymal Stem Cell Transplantation in Mice with Spinal Cord Injury. *Biomed Res Int* 2020;2020:7650354.
- [38] Marklein RA, Klinker MW, Drake KA, Polikowsky HG, Lessey-Morillon EC, Bauer SR. Morphological profiling using machine learning reveals emergent subpopulations of interferon- γ -stimulated mesenchymal stromal cells that predict immunosuppression. *Cytotherapy* 2019;21(1):17–31.