ORIGINAL ARTICLE

Veterinary Research Forum. 2024; 15 (1): 27 - 34
doi: 10.30466/vrf.2023.2003942.3903
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Immediate administration of hTERT-MSCs-IDO1-EVs reduces hypoalbuminemia after spinal cord injury

Shiva Amanollahi[1], Ahmad Reza Bahrami[2,3], Azadeh Haghighitalab[2,4], Hanieh Shaterzadeh Yazdi[5], Hossein Kazemi Mehrjerdi[1]*

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; [2] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran; [3] Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran; [4] Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR), Mashhad, Iran; [5] Private Veterinary Practitioner, Mashhad, Iran.

Article Info

Article history:

Received: 09 June 2023
Accepted: 23 August 2023
Available online: 15 January 2024

Keywords:

Extra-cellular vesicles
Glucose
Hypoalbunemia
Indoleamine 2,3-dioxygenase-1
Spinal cord injury

Abstract

Spinal cord injury (SCI) presents challenging and unpredictable neurological recovery. During inflammatory conditions, the amount of serum albumin and nutrition consumption decreases. Currently, it is proposed to measure serum albumin and glucose content in human or animal subjects to predict the recovery rate and the efficiency of treatments following SCI. In this study, the effect of extra-cellular vesicles (EVs) from immortalized human adipose tissue-derived mesenchymal stem cells (hTERT-MSCs) equipped with the ectopic expression of the human *indoleamine 2,3-dioxygenase-1* (*IDO1*) gene on serum albumin and glucose levels was investigated. After pre-clearing steps of 72-hr conditioned media, small EVs (sEVs) were isolated based on the ultra-filtration method. They were encapsulated with a chitosan-based hydrogel. Five experimental groups (female rats, N = 30, ~ 230 g) were considered, including SCI, sham, hydrogel, control green fluorescent protein (GFP)-EVs and *IDO1*-EVs. The 60.00 µL of hydrogel or hydrogels containing 100 µg sEVs from GFP or *IDO1*-EVs were locally injected immediately after SCI (laminectomy of the T10 vertebra and clip compression). After 8 weeks, non-fasting serum glucose and albumin levels were measured. The results indicated that the level of serum albumin in the animals received *IDO1*-EVs (3.52 ± 0.04) was increased in comparison with the SCI group (3.00 ± 0.94). Also, these animals indicated higher glucose levels in their serum (250.17 ± 69.61) in comparison with SCI ones (214 ± 45.34). Although these changes were not statistically significant, they could be considered as evidence for the beneficial effects of *IDO1*-EVs administration in the context of SCI to reduce hypoalbuminemia and improve energy consumption. More detailed experiments are required to confirm these results.

Introduction

Complications in the secondary phase of spinal cord injury (SCI) include a series of inflammatory responses, glial scar formation, site-specific axonal degeneration[1] and organ dysfunctions, as well as motor, sensory, urinary and autonomic nerve disorders below the injury site.[2] It is relatively common for patients or animals with SCI to experience pressure sores, infections, and nutritional changes such as malnutrition, over-nutrition, protein-energy malnutrition and energy failure. These factors make individuals more susceptible to hypoalbuminemia (HA) and reduced metabolic rate.[3] Following injury, spinal cord-specific cellular components such as enzymes and proteins are released into the bloodstream and cause hematological and biochemical changes.[4] Therefore, the measurement of hematological and biochemical markers, such as albumin, can potentially be used to predict functional recovery and effectiveness of the therapeutic interventions.[5-7]

The most abundant protein in blood plasma is albumin, which primarily acts as a blood transporter and helps maintain normal plasma oncotic pressure.[8] Albumin is proposed as a suitable marker for assessing malnutrition and certain inflammatory or chronic diseases.[9]

There are several factors that can cause HA (abnormal and low concentration of blood albumin). These factors include malnutrition, reduced synthesis due to liver

***Correspondence:**

Hossein Kazemi Mehrjerdi. DVM, PhD
Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
E-mail: h-kazemi@um.ac.ir

dysfunction, and increased catabolism following infection. Also, inflammatory status may lead to the leakage of albumin from the vascular system to extra-vascular spaces, which in turn increases trans-capillary escape rate of albumin. In addition, the leakage of plasma from the ulcer site in the form of sweat causes albumin loss.[10] Studies have shown that HA plays an important role in predicting morbidity and mortality in major diseases and various clinical situations.[11] It has also been reported that more severe SCI and poor neurological recovery can be associated with lower concentrations of serum albumin soon after the injury.[5] In addition, HA may reflect hyper-inflammation, reduced nutrition, and a decline in vital functions. It may also be due to the hyper-metabolic state associated with SCI, which may be exacerbated after surgical procedures.[12] However, the exact relationship between HA and poor outcomes has not yet been fully elucidated.

Today, the treatment of SCI remains one of the challenges for clinicians, and the effectiveness of common drugs is still controversial.[13] Cell transplantation is considered as a novel therapeutic approach to manage diverse complications associated with SCI.[14] Mesenchymal stem cells (MSCs) are multi-potent cells that can be isolated from several sources, including bone marrow, adipose tissue, umbilical cord, etc.[15] Among these sources, adipose tissue is more accessible during liposuction[16] and its collection is accompanied by minimal pain and invasiveness. These cells have anti-inflammatory effects and secrete different cytokines and growth factors which are important mediators of the immune system's function. Indoleamine 2,3-dioxygenase-1 (*IDO1*), nitric oxide (NO), prostaglandin-E2, transforming growth factor beta 1 (TGF-β1),[16] hepatocyte growth factor and interleukin-10 have been identified as the most efficient ones.[17] The *IDO1* is the rate-limiting enzyme in the tryptophan catabolic pathway and regarded as one of the key modulators of acquired immune tolerance.[18] The immunomodulatory role of *IDO1* has been reported in several publications.[19]

Although, the application of MSCs is associated with some limitations, such as the risk of chromosomal and genomic instability, potential risk of malignancies and altered functional properties.[20] Additionally, MSCs cannot cross the blood-brain barrier to reach the lesion site.[21] Over the past decades, these limitations have led cell-free therapies to replace cell-based therapies. Cell-free therapies can be obtained by applying active components of the stem cells' supernatant, mainly their extra-cellular vesicles (EVs).[22] The application of EVs as one of the main mediators of the paracrine effects of MSCs has gained special interest.[23] The EVs are biological nanoscale spherical lipid bilayer vesicles being secreted by most cell types. Exosomes are one of the main EV sub-types, with a diameter of 40.00 - 100 nm.[24] The EVs are classified based on different criteria, among which their size and biogenesis are more common. [25] Here, in accordance with the International Society for Extra-cellular Vesicles guidelines, the term small EVs (sEVs) is used to define the sub-population of EVs we applied following the SCI induction.[26] Due to their nanoscale dimensions, these membranous vesicles can easily cross the blood-spinal cord barriers and therefore, exert their immuno-modulatory properties, promote axonal regeneration and reduce neuroinflammation.[27]

Various administration routes including intravenous, intrathecal and local injections have been proposed to deliver EVs to the lesion site. Hydrogels have been suggested as ideal scaffolds for use in spinal cord contusions because they can replicate the mechanical properties of natural spinal cord tissue.[28] Moreover, their application may increase the durability of EVs at the site of injury, and will guarantee the slow and stable release of their contents.[21, 27, 28]

In this study, we aimed to determine if administering MSCs-EVs enriched from the conditioned medium (CM) of human telomerase reverse transcriptase (hTERT)-MSCs-IDO1 could decrease HA and increase the energy source in the plasma of the animal model. This is important considering the proposed beneficial effects of MSCs-EVs administration following SCI and the prognostic value of serum albumin and glucose concentrations.

Materials and Methods

Preparation of modified hTERT-MSCs. In this study, hTERT-immortalized adipose tissue-derived MSCs were used,[29] in which the human *IDO1* gene was over-expressed based on the lentiviral approach. Cells which were transduced with the backbone plasmid lacking the *IDO1* gene as the insert, hTERT-MSCs-green fluorescent protein (GFP), were used as a control for the main experimental group (hTERT-MSCs-IDO1).[30] Dulbecco's Modified Eagle Medium (DMEM)/F12 Medium (Gibco, Paisley, Scotland) containing puromycin (1.00 µg mL^{-1}; BioBasic, Toronto, Canada) was applied as a basal medium following the transduction steps (humidified incubator, 5.00% CO_2, 37.00 ˚C; Memmert, Schwabach, Germany). Cells were seeded in T175 cell culture flasks, and upon reaching the appropriate confluency (90.00 - 95.00%), they were sub-cultured (trypsin/ethylenediaminetetraacetic acid 0.25%; Gibco, Darmstadt, Germany) or stored for further applications. Control GFP or *IDO1* transduced cells were selected to be used as EV producing cells. The preparation of genetically engineered hTERT-MSCs was performed in accordance with the ethical guidelines of the National Institute for Medical Research Development. These guidelines were aligned with the World Medical Association Declaration of Helsinki (Ethics Code: IRNIMADREC1396040).

Ultra-filtration. The hTERT-MSCs-GFP and hTERT-MSCs-IDO1 were seeded in equal amounts in T175 cell culture flasks (2.00 × 10^6 cells *per* flask; SPL, Pocheon-si, South Korea). 48 hr later, their medium was removed and the basal DMEM/F12 medium supplemented with Exo-depleted Fetal Bovine Serum (10.00%)[31] was added to the culture vessels following two rounds of washing with phosphate-buffered saline (PBS; 1.00X). At two intervals of 72 hr, the CM was collected, pre-cleared with centrifugation (400 *g* for 6 min and 1,500 *g* for 30 min, 4.00 ˚C; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and syringe filtration (0.45 and 0.22 µm filters), and pooled. The prepared CM was used for sEV isolation immediately; otherwise, it was kept at –80.00 ˚C freezer. Isolation of sEVs (30.00 - 200 nm) was performed based on the application of a home-made ultra-filtration device. The ultra-filtration device, filtration-based device for exosome isolation from biological samples, being applied in the current project was registered in the Intellectual Property Center of Iran under Declaration Number Of 140050140003008723 on 7 February 2022 (Patent No. 108718, International Classification C12M 1/12; C12M 1/00). The isolated EVs were directly applied for downstream characterization steps or animal studies. Under aseptic conditions, 100 µg of IDO1 or GFP-EVs were dissolved in a hydroxyl ethyl cellulose solution (Fluka, Seelze, Germany) and then added to a mixture of β-glycerol phosphate (Sigma Aldrich, Darmstadt, Germany) and chitosan (Polysciences, Warrington, UK) while kept on ice.[32] Hydrogel containing insulin syringes or syringes filled with sEV-containing hydrogels (60.00 µL *per* insulin syringe *per* rat) were prepared and stored under cold aseptic conditions to be administered at the site of rat SCI during surgery procedures.

Bicinchoninic acid (BCA) assay. To determine the protein content of sEV preparations, the BCA assay was used according to the kit's instructions (Parstous, Mashhad, Iran). A serial dilution of bovine serum albumin was applied as a standard for protein concentrations. Optical densities were measured at 545 nm after a 60 min incubation at 60.00 ˚C using an ELISA Reader (Awareness Technology, Palm City, USA).

Dynamic light scattering (DLS). Particle size analysis (PSA) was performed based on DLS. Size distribution of samples was measured by a particle size analyzer (Vasco 3, Cordouan Technologies, Cité de la Photonique, France).

Zeta potential analysis. Zeta potential was measured to investigate the stability and physicochemical properties of the samples (Zeta Compact; CAD Instruments, Naucelle, France). To perform the measurement, the samples were diluted (1:80) in PBS and the measurement was conducted at pH of 7.40 and room temperature. Each sample was subjected to at least three independent measurements.

Induction of SCI. All ethical principles were considered during the animal studies and approved by the Ethics Committee of Ferdowsi University of Mashhad, Mashhad, Iran (Ethics Code: IR.UM.RES.1401.024). The Ethics Code is available on the website of the Iran National Committee for Ethics in Biomedical Research. In this study, 30 female *Wistar* rats (age: 12 weeks and weight: 200 - 250 g) were randomly divided into five groups including SCI, sham, hydrogel, hTERT-MSCs-GFP-EVs and hTERT-MSCs-IDO1-EVs. The rats were anesthetized using an intramuscular injection of ketamine (70.00 mg kg^{-1}; Bremer Pharma GmbH, Warburg, Germany) and xylazine (5.00 mg kg^{-1}; Alfasan, Woerden, The Netherlands). To establish the animal model, we followed a previously published protocol with minor changes. Briefly, after clipping the hair on the rats' back and surgically preparing the area, a 2.00 cm midline incision was made on the spinous process of T9-T10 vertebrae using a scalpel blade. The soft tissue and spinous process of T10 vertebra were removed. Laminectomy of T10 vertebra was performed to fully access the spinal cord. The drake aneurysm clip (Codman, Sheffield, UK) was placed on the spinal cord for one min.[33] Injection of 60.00 µL hydrogel or 60.00 µL hydrogel containing 100 µg sEVs was performed over the injured site immediately following the induction of SCI. The sham group only received laminectomy without SCI and did not receive any drugs (Fig. 1). Then, the muscles and skin were sutured using 3-0 vicryl (Supa, Tehran, Iran). Post-operative care includes the administration of 3.00 mL Ringer's solution (Samen Co., Mashhad, Iran) subcutaneously, intramuscular injection of tramadol (Darou Pakhsh Pharmaceutical Co., Tehran, Iran) at a dose of 10.00 mg kg^{-1} every 12 hr for three days and subcutaneous injection of diluted enrofloxacin (Razak, Tehran, Iran) at a dose of 10.00 mg kg^{-1} every 12 hr for one week.

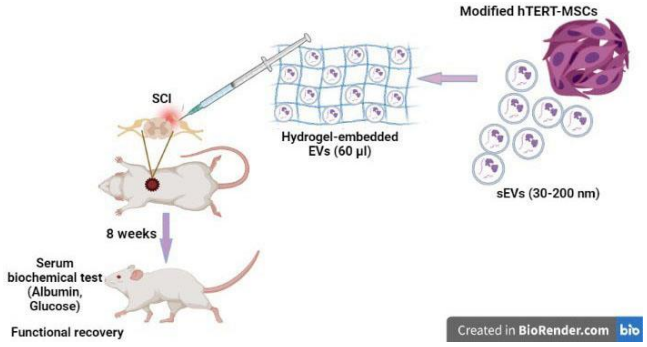

Fig. 1. Schematic representation of the study. Small extra-cellular vesicles (sEVs) derived from hTERT-MSCs-GFP and hTERT-MSCs-IDO1 were embedded in modified chitosan hydrogel and injected at the site of injury (60.00 µL hydrogel or 60.00 µL hydrogel containing 100 µg sEVs) immediately following spinal cord injury (SCI) induction. After 8 weeks, the rats were sacrificed and their serum samples were used for downstream biochemical analysis measuring serum albumin and non-fasting glucose levels (created by BioRender.com). GFP: Green fluorescent protein; hTERT: Human telomerase reverse transcriptase; IDO1: Indoleamine 2,3 dioxygenase; MSCs: Mesenchymal stem/stromal cells.

Bladder emptying through massage was performed twice a day until the return of bladder reflex activity, and physiotherapy and foot massage were also performed daily until the movement of the joints returned. The rats were maintained for 8 weeks in suitable conditions with free access to food and water.

Serum albumin and glucose measurement. After 8 weeks, under deep general anesthesia, intracardiac blood samples of the rats were taken under aseptic conditions and placed in plain tubes for serum separation. All tubes were immediately placed on ice and transferred to the laboratory. The plain tubes were centrifuged at 3,000 *g* for 10 min followed by removal of the serum. The serum was stored at – 80.00 ˚C until biochemical analyses. Finally, non-fasting glucose and albumin levels were measured using commercial kits (Pars Azmoon, Tehran, Iran) and an autoanalyser device (Targa 3000; Biotecnica, Rome, Italy).

Statistical analyses. The results were reported as mean ± standard deviation using SPSS Software (version 26.0; IBM Corp., Armonk, USA). Normality was assessed with the Kolmogorov-Smirnov test. Serum glucose was analyzed with one-way ANOVA and Tukey's multiple comparisons *post hoc* test. Serum albumin was analyzed using the Mann-Whitney U test. A significance level of $p < 0.05$ was considered significant.

Results

Isolation and characterization of hTERT-MSCs-IDO1-EVs. The hTERT-MSCs-IDO1 and control hTERT-MSCs-GFP cell lines, which were used as EV producer cells were previously established[30] and their characteristics were determined based on the minimal criteria recommended by the International Society for Cell and Gene Therapy.[34] Figure 2 shows microscopic images of hTERT-MSCs before and after the transduction steps. As mentioned in the materials and methods, to isolate sEVs, the ultra-filtration device was applied following the pre-clearing steps of pooled CM from each experimental group.

The number of cells at the time of CM harvest was determined to be 2.70×10^5 based on the formula suggested by Börger *et al.*[35] The total protein content of sEVs was determined to be 6008.78 µg mL^{-1} for GFP-EVs and 7528.72 µg mL^{-1} for IDO1-EVs based on the BCA assay. These concentrations were applied as a quantification criterion to normalize the amount of sEVs administered to each rat during injections. The size distribution of the particles was determined to be between 30.00 - 200 nm with number distribution size of 47.28, Z-average/harmonic intensity averaged particular diameter of 179.98, and poly dispersity index of 0.3500 *via* PSA analysis (Fig. 3A). Zeta potential analysis reported a mean zeta potential of – 25.07 ± 8.06 mV, indicating an acceptable level of stability for IDO1-EVs (temperature: 24.15 ˚C and pH: 7.80; Fig. 3B).

Serum concentration of albumin. Evaluating the concentration of albumin in the blood serum of rats 8 weeks following the surgeries indicated that the amount of serum albumin in the SCI group was lower than other groups. This decreasing trend in serum albumin levels was observed in the hydrogel, GFP-EVs and IDO1-EVs groups, respectively (Table 1). However, these differences were not statistically significant ($p > 0.05$).

Table 1. Comparison of serum albumin and glucose concentrations between different groups at 8 weeks post-surgery.

Experimental groups	Albumin (g dL^{-1})	Glucose (mg dL^{-1})
SCI	3.00 ± 0.94	214.00 ± 45.34
Sham	3.63 ± 0.21	246.50 ± 36.72
Hydrogel	3.28 ± 0.21	220.17 ± 55.69
MSCs-GFP-EVs	3.48 ± 0.19	232.00 ± 31.44
MSCs-*IDO1*-EVs	3.52 ± 0.04	250.17 ± 69.61

EVs: Extra-cellular vesicles; GFP: Green fluorescent protein; IDO1: Indoleamine 2,3 dioxygenase; MSCs: Mesenchymal stem/stromal cells; SCI: Spinal cord injury.
No significant difference was observed between groups in terms of serum albumin and glucose levels ($p > 0.05$).

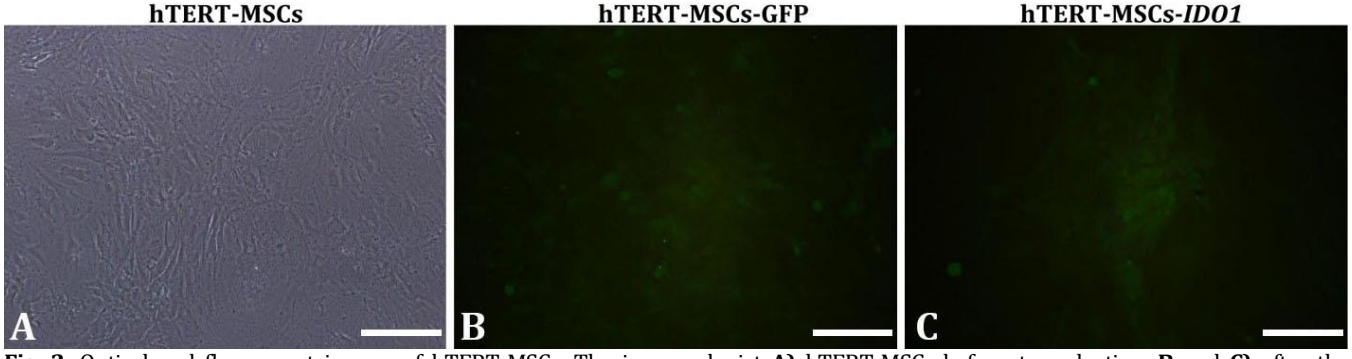

Fig. 2. Optical and fluorescent images of hTERT-MSCs. The images depict **A)** hTERT-MSCs before transduction, **B** and **C)** after the transduction steps. After the lentiviral transduction and the initial selection (2.00 µg mL^{-1} puromycin, 72 hr), green fluorescent cells were maintained under a lower concentration of antibiotic for an additional 15 days. The hTERT-MSCs-GFP received lentiviral particles without *IDO1* as an insert (Olympus, Tokyo, Japan; bars = 200 µm). GFP: Green fluorescent protein; hTERT: Human telomerase reverse transcriptase; IDO1: Indoleamine 2,3 dioxygenase; MSCs: Mesenchymal stem/stromal cells.

Serum concentration of glucose. As shown in the Table 1, the SCI group had the lowest blood glucose level. Following that, the hydrogel and GFP-EVs groups had the lowest blood glucose levels, respectively. On the other hand, the IDO1-EVs group had the highest blood glucose level, even higher than the sham group. However, no statistically significant difference was observed ($p > 0.05$). The hTERT-MSCs-IDO1-EVs decreased HA in comparison with SCI, hydrogel and GFP-EVs groups. Serum glucose level in IDO1-EVs group was higher than other experimental groups.

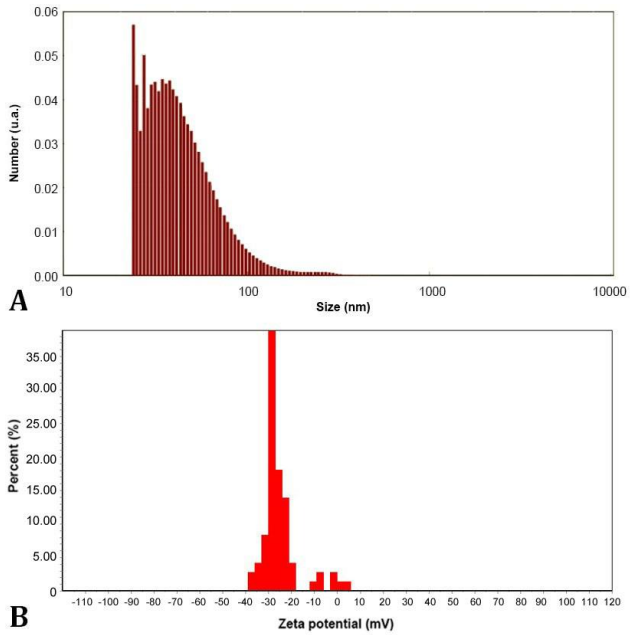

Fig. 3. Particle size and zeta potential analyses. **A)** Particle size analysis based on dynamic light scattering confirmed desirable size range for extra-cellular vesicles (EVs) isolated based on the ultra-filtration method (mean: 47.28). **B)** Mean of zeta potential of – 25.07 mV was recorded for *indoleamine 2,3 dioxygenase*-EVs.

Discussion

Primary SCI causes axonal damage and tissue necrosis. Following SCI, a cascade of secondary events including metabolic, biochemical and cellular changes, such as electrolyte abnormalities, lipid peroxidation, free radicals formation, NO action, ischemia, edema and apoptosis in the microcirculation occurs.[36] Additionally, there is an increased secretion of pro-inflammatory cytokines after SCI, leading to increased vascular permeability, dilution of serum albumin, accelerated removal of albumin from the circulation, and HA development. It is believed that HA may indicate a hostile pro-inflammatory state,[37] and could be a strong predictor of poor outcomes for various central nervous system (CNS) diseases and injuries, including traumatic brain[38] and chronic SCIs.[12] In patients with neurological damage, persistent reduction in albumin levels leads to organ function destruction, impairs recovery from spinal cord edema, and exacerbates blood supply, nutrition and metabolism of the nervous system. These events perpetuate a debilitating cycle.[39] On the other hand, several studies have demonstrated the neuroprotective role of albumin in CNS injuries.[40]

The relationship between albumin, inflammation and neurological recovery has also been described in a previous study.[5] As one of the main therapeutic goals after SCI is to modulate the inflammatory environment to promote neuronal regeneration and manage extensive secondary cell death, we used genetically modified hTERT-MSCs to modulate inflammatory events in a rat model of SCI. These cells retain essential features of primary cultures of MSCs including spindle-like morphology, adherence to tissue culture plastic, differentiation and growth capacity over several passage numbers, serving as an immortalized model cell line of MSCs.[29] Moreover, due to their origin, it can be assumed that they will have prominent immuno-regulatory properties reported for primary cultures of MSCs derived from adipose tissue.[41] Additionally, hTERT immortalization has previously been suggested as a practical method for establishing of cell lines with a remarkable ability to prepare clinical-grade EVs.[42]

In a previous study, we investigated the immune regulatory characteristics of hTERT-MSCs and their EV counterparts against human peripheral blood mono-nuclear cells (hPBMCs) and Jurkat cells. The cells were primed with different compounds or genetically modified using the lentiviral approach to over-express human *IDO1*, *prostaglandin-endoperoxide synthase* and *TGF-β1* genes individually or concurrently in the co-transduced group. Results indicated that hTERT-MSCs-IDO1-EVs showed the highest proliferation inhibitory effects against allogenic hPBMCs.[30] Different catabolites, closely related to various diseases, are involved in the formation of the immunosuppressive environment, and different mechanisms have been proposed to describe the *IDO1*-mediated immunoregulation. This immunoregulation may be due to the lack of local tryptophan in the micro-environment, activating the general control nonderepressible 2 (GCN2) kinase and mammalian target of rapamycin pathways, as well as T cell anergy.[43] Consumption of tryptophan by *IDO1* removes this essential amino acid required for the production of various proteins in the environment. Tryptophan deficiency activates GCN2 kinase in T cells leading to cell arrest in the G2 phase of the cell division cycle. Inhibition of T helper type 1 (Th1) cells by *IDO1* causes a selective increase of Th2 cells response.[44] Additionally, when $CD4^+$ T cells combine with *IDO1*, they become regulatory T (Treg) cells, inhibiting the activation, differentiation and survival of killer T cells. The Treg cell response induced by *IDO1* inhibits both types of Th1 and Th2 cells; thereby, moderating the immune system. Moreover, *IDO1* metabolites are capable of inhibiting the proliferation and

function of natural killer cells. All these factors create an environment that helps to adjust and regulate the immune system. [45]

Here, we hypothesize that hTERT-MSCs-IDO1-EVs may prevent the spread of secondary damage following SCI by modulating the immune system's activity. This is based on their anti-inflammatory and immuno-modulatory properties, controlling the inflammatory environment and inhibiting the inflammatory factors expression.[46] As a proof of concept, we demonstrated that the immediate administration of hTERT-MSCs-IDO1-EVs in a xenogeneic rat model of SCI improves the functional recovery of rats in comparison with other experimental groups (data not shown). Additionally, hTERT-MSCs-IDO1-EVs are indicated to reduce HA and improve nutrition after SCI.

The beneficial effects of albumin in the CNS include the reduction of free radicals, platelet aggregation, leukocyte adhesion and histological damage to neurons, prevention of capillary stasis and NO regulation. Moreover, albumin has been shown to improve metabolic activity of neurons and micro-circulatory flow in animal studies.[39] Recent studies have also shown that prolonged HA is proportional to a lesser neurological disease,[5,7] inflammation, infection, chronic diseases,[47] patient malnutrition[9] and poor health, as well as an increased risk of mechanical ventilation and mortality after acute SCI.[39] It has been reported that intra-peritoneal treatment with human serum albumin improves early clinical results, protects spinal cord ultra-structure, and decreases myeloperoxidase activity and lipid peroxidation levels after spinal cord contusion injury in rats in comparison with methylprednisolone.[40]

Leister *et al*. have shown that routine blood chemistry data from post-acute phase, along with baseline injury severity can predict functional outcome after incomplete SCI.[37] Also, Vo *et al*. and Jin *et al*. have demonstrated that albumin concentration correlates with baseline characteristics and long-term neurological recovery after SCI. During rehabilitation, serum albumin concentration can also estimate the severity of injury and future neurological recovery.[7, 39] In a study by Tong *et al*., the effect of monosialotetrahexo-sylganglioside on human serum albumin levels and motor performance improvement was investigated. It was found that higher serum albumin concentrations at 1, 2 and 4 week periods were associated with higher 52-week motor function scores. The researchers suggested that serum albumin could serve as a feasible biomarker for prognosis at the time of injury and stratification in clinical trials.[5]

The main source of energy for the CNS is glucose, which ensures the necessary energy for neural activity.[48] Disturbances in glucose metabolism lead to the occurrence and development of various diseases related to the nervous system.[49] It has been concluded that remarkable reduction of whole body glucose is mainly due to the reduction in muscle mass (atrophy), and that skeletal muscles have a remarkable capacity to maintain their glucose transport system intact even in the face of severe denervation and morphological changes.[50]

In the present study, the glucose levels were measured in a non-fasting state to monitor its levels under normal conditions. Similar to serum albumin levels, the SCI group showed the lowest blood glucose level compared to the other groups. The hydrogel and GFP-EVs groups had higher serum glucose levels than SCI group; but, lower than the IDO1-EVs group. We hypothesize that the lack of improvement in the nervous system in the absence of any treatment schedule and the subsequent decrease in the movement capacity/score along with factors such as infection and stress may lead to a decrease in serum albumin and blood glucose levels. However, further investigations are required to attribute these results to the human cases.

Biochemical parameters can be assessed repeatedly in the first days or weeks after SCI without the need for invasive procedures. This evaluation is quick and inexpensive. The levels of these parameters are directly related to the severity of injury and the extent of neurological recovery. In our study, we utilized the measurement of serum albumin and glucose levels to assess the effectiveness of hTERT-MSCs-IDO1-EVs in a rat model of SCI. We propose that EVs derived from the CM of hTERT-MSCs-IDO1 can replicate the immuno-modulatory characteristics of their parent cells and their anti-inflammatory effects can be detected through simple blood tests.

Acknowledgments

The authors thank the staff of the Faculty of Veterinary Medicine Teaching Hospital of Ferdowsi University of Mashhad, as well as the Cell Culture Laboratory of Ferdowsi University of Mashhad, Mashhad, Iran, for their valuable technical cooperation. Additionally, we extend our thanks to the members of the Stem Cells and Regenerative Medicine Department at the Academic Center for Education, Culture and Research, Khorasan Razavi Branch, Mashhad, Iran. Special thanks go to Halimeh Hassanzadeh, Dr. Mahboubeh Kazemi-Noughabi and Dr. Moein Farshchian, who were involved in the establishment of genetically modified cells. This research was supported by the Research Council of the Faculty of Veterinary Medicine (Grant No. 57497) and Faculty of Science (Grant No. 41827) of Ferdowsi University of Mashhad, Mashhad, Iran. Additionally, this project received partial support from the National Institute for Medical Research Development of Iran (Grant No. 957797). Azadeh Haghighitalab was also partially supported by a grant from Ferdowsi University of Mashhad, Mashhad, Iran (No. FUM-14002794075).

Conflict of interest

The authors declare no conflict of interest.

References

1. Wertheim L, Edri R, Goldshmit Y, et al. Regenerating the injured spinal cord at the chronic phase by engineered iPSCs-derived 3D neuronal networks. Adv Sci (Weinh) 2022; 9(11): e2105694. doi: 10.1002/advs.202105694.
2. Craig A, Perry KN, Guest R, et al. Prospective study of the occurrence of psychological disorders and comorbidities after spinal cord injury. Arch Phys Med Rehabil 2015; 96(8): 1426-1434.
3. Zeng X, Xiang J, Dong L, et al. Advance in nutritional status and intervention after spinal cord injury. J Clin Nurs Res 2021; 5(6): 7-14.
4. Bourguignon L, Vo AK, Tong B, et al. Natural progression of routine laboratory markers following spinal trauma: a longitudinal, multi-Cohort study. J Neurotrauma 2021; 38(15): 2151-2161.
5. Tong B, Jutzeler CR, Cragg JJ, et al. Serum albumin predicts long-term neurological outcomes after acute spinal cord injury. Neurorehabil Neural Repair 2018; 32(1): 7-17.
6. Brown SJ, Harrington GMB, Hulme CH, et al. A preliminary Cohort study assessing routine blood analyte levels and neurological outcome after spinal cord injury. J Neurotrauma 2020; 37(3): 466-480.
7. Vo AK, Geisler F, Grassner L, et al. Serum albumin as a predictor of neurological recovery after spinal cord injury: a replication study. Spinal Cord 2021; 59(3): 282-290.
8. Fanali G, di Masi A, Trezza V, et al. Human serum albumin: from bench to bedside. Mol Aspects Med 2012; 33(3): 209-290.
9. Marcason W. Should albumin and prealbumin be used as indicators for malnutrition? J Acad Nutr Diet 2017; 117(7): 1144. doi: 10.1016/j.jand.2017.04.018.
10. Gatta A, Verardo A, Bolognesi M. Hypoalbuminemia. Intern Emerg Med 2012; 7(Suppl 3): S193-S199.
11. Kim SD, Melikian R, Ju KL, et al. Independent predictors of failure of nonoperative management of spinal epidural abscesses. Spine J 2014; 14(8): 1673-1679.
12. Frisbie JH. Anemia and hypoalbuminemia of chronic spinal cord injury: prevalence and prognostic significance. Spinal Cord 2010; 48(7): 566-569.
13. Khodabakhshi Rad A, Kazemi Mehrjerdi H, Pedram MS, et al. Clinical evaluation of the effect of methyl-prednisolone sodium succinate and meloxicam in experimental acute spinal cord injury. Iran J Vet Med 2023; 17(2):129-138. doi: 10.32598/IJVM. 17.2.1005246.
14. Feng J, Zhang Y, Zhu Z, et al. Emerging exosomes and exosomal MiRNAs in spinal cord injury. Front Cell Dev Biol 2021; 9: 703989. doi: 10.3389/fcell.2021.703989.
15. Sykova E, Cizkova D, Kubinova S. Mesenchymal stem cells in treatment of spinal cord injury and amyotrophic lateral sclerosis. Front Cell Dev Biol 2021; 9: 695900. doi: 10.3389/fcell.2021.695900.
16. Navajas R, Corrales FJ, Paradela A. Serum exosome isolation by size-exclusion chromatography for the discovery and validation of preeclampsia-associated biomarkers. Methods Mol Biol 2019; 1959: 39-50.
17. Ge W, Jiang J, Arp J, et al. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2, 3-dioxygenase expression. Transplantation 2010; 90(12):1312-1320.
18. Meireson A, Devos M, Brochez L. IDO expression in cancer: different compartment, different functionality? Front Immunol 2020; 11: 531491. doi: 10.3389/ fimmu.2020.531491.
19. Proietti E, Pauwels RWM, de Vries AC, et al. Modulation of indoleamine 2, 3-dioxygenase 1 during inflammatory bowel disease activity in humans and mice. Int JTryptophan Res 2023; 16: 11786469231153109. doi: 10.1177/11786469231153109.
20. Lukomska B, Stanaszek L, Zuba-Surma E, et al. Challenges and controversies in human mesenchymal stem cell therapy. Stem Cells Int 2019; 2019: 9628536. doi: 10.1155/2019/9628536.
21. Zhang X, Jiang W, Lu Y, et al. Exosomes combined with biomaterials in the treatment of spinal cord injury. Front Bioeng Biotechnol 2023; 11: 1077825. doi: 10.3389/fbioe.2023.1077825.
22. Hartjes TA, Mytnyk S, Jenster GW, et al. Extracellular vesicle quantification and characterization: common methods and emerging approaches. Bioengineering (Basel) 2019; 6(1): 7. doi: 10.3390/bioengineering 6010007.
23. Mathieu M, Martin-Jaular L, Lavieu G, et al. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 2019; 21(1): 9-17.
24. Pluchino S, Smith JA. Explicating exosomes: reclassifying the rising stars of intercellular communication. Cell 2019; 177(2): 225-227.
25. Zhang C, Deng R, Zhang G, et al. Therapeutic effect of exosomes derived from stem cells in spinal cord injury: a systematic review based on animal studies. Front Neurol 2022; 13: 847444. doi: 10.3389/fneur. 2022.847444.
26. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018; 7(1): 1535750. doi: 10.1080/20013078.2018.1535750.

27. Khalatbary AR. Stem cell-derived exosomes as a cell free therapy against spinal cord injury. Tissue Cell 2021; 71: 101559. doi: 10.1016/j.tice.2021.101559.
28. Wang K, Dong R, Tang J, et al. Exosomes laden self-healing injectable hydrogel enhances diabetic wound healing via regulating macrophage polarization to accelerate angiogenesis. Chem Eng J 2022; 430: 132664. doi: 10.1016/j.cej.2021.132664.
29. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy 2006; 8(4): 315-317.
30. Börger V, Staubach S, Dittrich R, et al. Scaled isolation of mesenchymal stem/stromal cell-derived extracellular vesicles. Curr Protoc Cell Biol 2020; 55(1): e128. doi: 10.1002/cpsc.128.
31. Leister I, Mittermayr R, Mattiassich G, et al. The effect of extracorporeal shock wave therapy in acute traumatic spinal cord injury on motor and sensory function within 6 months post-injury: a study protocol for a two-arm three-stage adaptive, prospective, multicenter, randomized, blinded, placebo-controlled clinical trial. Trials 2022; 23(1): 245. doi: 10.1186/s13063-022-06161-8.
32. Tátrai P, Szepesi Á, Matula Z, et al. Combined introduction of Bmi-1 and hTERT immortalizes human adipose tissue-derived stromal cells with low risk of transformation. Biochem Biophys Res Commun 2012; 422(1): 28-35.
33. Haghighitalab A, Matin MM, Amin A, et al. Investigating the effects of *IDO1*, *PTGS2*, and *TGF-β1* overexpression on immunomodulatory properties of hTERT-MSCs and their extracellular vesicles. Sci Rep 2021; 11: 7825. doi: 10.1038/s41598-021-87153-7.
34. Haghighitalab A, Matin MM, Khakrah F, et al. Cost-effective strategies for depletion of endogenous extracellular vesicles from fetal bovine serum. J Cell Mol Res 2020; 11(2): 42-54.
35. Naderi-Meshkin H, Andreas K, Matin MM, et al. Chitosan-based injectable hydrogel as a promising in situ forming scaffold for cartilage tissue engineering. Cell Biol Int 2014; 38(1): 72-84.
36. Javdani M, Ghorbani R, Hashemnia M. Histopathological evaluation of spinal cord with experimental traumatic injury following implantation of a controlled released drug delivery system of chitosan hydrogel loaded with selenium nanoparticle. Biol Trace Elem Res 2021; 199(7): 2677-2686.
37. Leister I, Linde LD, Vo AK, et al. Routine blood chemistry predicts functional recovery after traumatic spinal cord injury: a post hoc analysis. Neurorehabil Neural Repair 2021; 35(4): 321-333.
38. Bernard F, Al-Tamimi YZ, Chatfield D, et al. Serum albumin level as a predictor of outcome in traumatic brain injury: potential for treatment. J Trauma 2008; 64(4): 872-875.
39. Jin GX, Li L, Cui SQ, et al. Persistent hypoalbuminemia is a predictor of outcome in cervical spinal cord injury. Spine J 2014; 14(9): 1902-8.
40. Yildirim T, Okutan O, Akpinar E, et al. Neuroprotective effects of high-dose human albumin against traumatic spinal cord injury in rats. Bratisl Lek Listy 2018; 119(2): 86-91.
41. Wang ZG, He ZY, Liang S, et al. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res Ther 2020; 11(1): 511. doi: 10.1186/s13287-020-02032-8.
42. Rohde E, Pachler K, Gimona M. Manufacturing andcharacterization of extracellular vesicles from umbilical cord–derived mesenchymal stromal cells for clinical testing. Cytotherapy 2019; 21(6): 581-592.
43. Zeitler L, Murray PJ. IL4i1 and IDO1: oxidases that control a tryptophan metabolic nexus in cancer. J Biol Chem 2023; 299(6): 104827. doi: 10.1016/j.jbc.2023.104827.
44. Murakami Y, Hoshi M, Imamura Y, et al. Remarkable role of indoleamine 2, 3-dioxygenase and tryptophan metabolites in infectious diseases: potential role in macrophage-mediated inflammatory diseases. Mediators Inflamm 2013; 2013: 391984. doi: 10.1155/2013/391984.
45. Mándi Y, Vécsei L. The kynurenine system and immunoregulation. J Neural Transm (Vienna) 2012; 119(2): 197-209.
46. Günther J, Fallarino F, Fuchs D, et al. Editorial: immunomodulatory roles of tryptophan metabolites in inflammation and cancer. Front Immunol 2020; 11: 1497. doi: 10.3389/fimmu.2020.01497.
47. Liu JM, Deng HL, Chen XY, et al. Risk factors for surgical site infection after posterior lumbar spinal surgery. Spine (Phila Pa 1976) 2018; 43(10): 732-737.
48. Scholpa NE, Schnellmann RG. Mitochondrial-based therapeutics for the treatment of spinal cord injury: mitochondrial biogenesis as a potential pharmacological target. J Pharmacol Exp Ther 2017; 363(3): 303-313.
49. Cisternas P, Salazar P, Silva-Álvarez C, et al. Activation of Wnt signaling in cortical neurons enhances glucose utilization through glycolysis. J Biol Chem 2016; 291(50): 25950-25964.
50. Giuliani G, Rosina M, Reggio A. Signaling pathways regulating the fate of fibro/adipogenic progenitors (FAPs) in skeletal muscle regeneration and disease. FEBS J 2022; 289(21): 6484-517.