Intranasal delivery of human oligodendrocyte precursor cells enhances regenerative effects in premature rat brain following hypoxic–ischemic injury

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Abstract

Background

White matter injury is a common ischemic brain injury in premature infants and for which there is no effective treatment. Stem cell transplantation has emerged as a novel approach for replacing damaged brain tissues and promoting regenerative processes. Since intracerebral transplantation is not clinically feasible for premature infants, intranasal delivery of cells to the brain is a promising, noninvasive therapeutic approach for restoring the damaged brain. This study was conducted to investigate whether transplanted human oligodendrocyte progenitor cells exhibit neuroprotective effects in a rat model of WMI in premature infants.

Methods

SD rats aged 3 days were randomly divided into sham group, preterm white matter injury group and nasal transplantation group. Hypoxia–ischemia was induced in 3-day-old rat pups by right carotid artery ligation, followed by exposure to 6% oxygen for 90 min. Rats were intranasally administered the cell suspension (250,000 cells in 6 μ L) twice in each nostril (1×10^6 cells total), and myelin basic protein immunofluorescence staining and transmission electron microscopy were performed to assess endogenous myelin growth in the right hemispheres. Behavioral tests, including the Morris water maze, adhesive-removal test, and cylinder rearing test, as well as a gait test, were performed to evaluate the therapeutic effects at 12 weeks after transplantation.

Results

The cells transplanted from the nose can enter brain tissue of rats 3 days after transplantation. The myelin sheath structure was more compact and the myelin sheath thickness was increased. The nerve function defect was improved 12 weeks after transplantation.

Conclusions

The intranasal administration of human oligodendrocyte progenitor cells had beneficial therapeutic effects on rehabilitation of the rat hypoxia-ischemia model. This technique is a potential strategy for applying oligodendrocyte progenitor cells in transplantation therapy against white matter injury.

Background

The human preterm brain is particularly susceptible to cerebral white matter injury (WMI), which disrupts the normal progression of developmental myelination. WMI remains the most common cause of sensorimotor disabilities, cognitive dysfunction, and learning and memory disorders in premature babies.

According to the World Health Organization, ~ 15 million preterm infants (1 in 10 live births) are born at < 37-weeks post-conceptional age annually worldwide, representing a leading cause of death in babies < 5 years of age [1]. The susceptibility to preterm WMI peaks at 23–32-weeks post-conception. WMI pathogenesis is related to three major interacting factors: cerebral hypoxia/ischemia, systemic infection/inflammation, and intrinsic vulnerability of cerebral white matter pre-oligodendrocytes (pre-OLs) during the peak period of WMI occurrence. Pre-OLs are the predominant population of pre-myelinating oligodendrocyte progenitors in preterm human cerebral white matter [2] and particularly susceptible to considerable oxidative damage, the magnitude of which is comparable to that observed in cerebral cortical hypoxic–ischemic lesions in infants [3].

Currently, efficacious therapeutic strategies to protect the developing brain against ischemic damage in premature babies are limited. In preclinical studies, hypothermia therapy showed good protection against white matter damage [4], and the combination of hypothermia therapy and drugs to prevent brain death is a potential method for WMI treatment in premature infants. However, clinical data on the safety of hypothermia treatment for preterm infants is insufficient, and many drugs have not been widely evaluated for WMI in preterm infants [5].

Preclinical studies showed that stem cell treatment effectively restores injured organs and tissues in animal models; the cell transplantation methods used in these models typically include arteriovenous transplantation, stereotactic brain transplantation, lumbar puncture, and lateral ventricle transplantation. These methods are invasive, complicated, and technically demanding and carry a procedural risk for the patient, thus making repeated treatment difficult and infeasible in the clinical setting for premature babies [6]. Therefore, a non-invasive, convenient, and effective transplantation approach is needed for wider clinical use in premature infants. The intranasal cavity provides a direct passage to the intracerebral compartment along the olfactory pathways and has been used to deliver drugs directly to the central nervous system (CNS), bypassing the blood–brain barrier and minimizing systemic exposure [7]. To target the brain, stem cells have also been applied via the nasal route [8], and cells can enter the naive murine brain following intranasal delivery [9].

Because of their restorative potential and inherent pathotropism, oligodendrocyte precursor cells (OPCs) are ideal candidates for targeted delivery of biologically-active gene products in many neurological disorders, including stroke, neurodegenerative disorders, and brain tumors; however, intranasal administration of OPCs to treat WMI in premature infants has not been examined. Therefore, this study was performed to investigate the feasibility of intranasal administration of OPCs as an alternative, non-invasive, and direct route for delivering stem cells to the target.

Materials And Methods

Human (h)OPC culture and CM-Dil labeling

Human fetal brain tissue was derived from embryos aged 10–13 weeks, and cells were prepared in the Pediatric Laboratory of the Sixth Medical Center of the General Hospital of the Chinese People's

Liberation Army (Beijing, China). Human fetal brain was mechanically dispersed, centrifuged, and separated to form a single-cell suspension, which was seeded into medium containing a cocktail of basic fibroblast growth factor, platelet-derived growth factor (PDGF), and neurotrophic factor-3 for the passage and amplification of neural stem cells [10]. Neural stem cells were induced to form OPCs to obtain relatively pure hOPCs [11]. Cells were cultured in a humidified atmosphere of 5% CO₂ and 95% air at 37°C, and hOPC purity was evaluated by immunofluorescence staining with specific markers [ganglioside (A2B5), neural/glial antigen 2 (NG2), PDGF receptor-α (PDGFR-α), SRY-box transcription factor 10 (SOX10), oligodendrocyte transcription factor 2 (Olig2), O1, glial fibrillary acidic protein (GFAP), and class III β-tubulin (Tuj-1)].

To better trace and observe the transplanted cells, hOPCs were labeled with Cell Tracker CM-Dil (40718ES50; YEASEN, Shanghai, China) prior to transplantation by incubating the cells with the dye for 5 min at 37°C and others for 15 min at 4°C. After labeling, the cells were washed with PBS and resuspended in hOPC medium [10]. OPC medium was prepared by adding 2% B27 supplement, 20 ng/mL basic fibroblast growth factor, 1% penicillin/streptomycin, 5 µg/mL heparin, and 2 mM L-glutamine to Neurobasal-A medium. The labeling efficiency of the cells was verified at >95% prior to all transplantations by fluorescence microscopy (OLYMPUS, Tokyo, Japan).

Establishment of the WMI model and intranasal delivery of hOPCs

All experimental procedures were approved by the Animal Ethical and Welfare Committee of the Sixth Medical Center of Chinese People's Liberation Army General Hospital (Beijing, China). Pregnant Sprague–Dawley rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). At postnatal day 3 (p3), Sprague–Dawley rat pups (7–9 g) were anesthetized with 4% chloral hydrate and underwent hypoxia–ischemia (HI) by permanent right common carotid artery occlusion, followed by exposure to 6% oxygen and 94% nitrogen at 37°C for 90 min before being returned to their dam. In the sham group, only the right common carotid artery was exposed without common carotid cutting and subsequent hypoxia. For intranasal delivery, rats were administered 100 U hyaluronidase (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile PBS 30 min before administration of the cells. A Hamilton microsyringe needle (Hamilton Co., Reno, NV, USA) was inserted into the plastic hose of the drip liquid, followed by intranasal delivery of 6-µL drops (24 µL total) of cell suspension (1×10^6 cells) twice to each nostril. Droplets were administered into alternating nostrils at 15-min intervals. During these procedures, the mouth and opposite nostrils were closed to allow the cell suspension to pass into the nasal cavity of the animal. At 3 days after HI, experimental animals were randomly assigned to three different experimental groups: sham group, preterm white matter injury (PWMI) group and nasal transplantation group ($n=3$). Eight days after HI, experimental animals were randomly assigned to three different experimental groups: sham group, preterm white matter injury group and nasal transplantation group ($n=10$). All rats were intraperitoneally injected with cyclosporine (10 mg/kg/day) 3 days before transplantation.

Immunohistochemical staining

Animals were transcardially perfused with 4% paraformaldehyde solution at 12 weeks after hOPC application and post-fixed in the same solution for 24 h, followed by cryoprotection in 30% sucrose for 72 h. The brain was perfused to obtain frozen sections (thickness: 8 μ m). After rewarming, the cells were washed three times in PBS for 5 min each. Coronal sections (8 μ m) were successively blocked in 0.3% Triton X-100 and 5% bovine serum for 1 h at 37°C, followed by incubation with a primary antibody specific for myelin basic protein (MBP; 1:100, rat monoclonal; Abcam, Cambridge, UK) at 4°C overnight followed by secondary antibodies conjugated with Alexa Fluor 488 (1:200; Molecular Probes, Eugene, OR, USA) for 2 h at 37°C. The slides were washed again with PBS before mounting with DAPI.

Transmission electron microscopy and g-ratio analysis

Right hemispheres were used for transmission electron microscopy (TEM) at 12 weeks post-transplantation. Brain sections (1–3-mm thick) from the right corpus callosum of rats (n = 3/group) were fixed in 2.5% glutaraldehyde and 2% formaldehyde at 4°C for 72 h and then stained with osmium tetroxide overnight. The sections were dehydrated in an ethanol series and embedded in epoxy resin. Ultrathin sections were observed by TEM (Hitachi, Tokyo, Japan) after electron staining, and images were captured for further analysis. We selected 100 myelin sheaths (6000 $\times$ visual field) from each sample to evaluate the myelin thickness (g-ratio) and classification, except in extremely long or distorted axons. Corpus callosum g-ratios were determined from the TEM images by manually measuring the axon diameter and outer myelin-layer diameter (axon average diameter/fiber average diameter) using Image-Pro Plus software (v.6.0; Media Cybernetics, Rockville, MD, USA).

Behavioral tests

Behavioral tests such as the Morris water maze test, adhesive-removal test, cylinder test, and gait analysis are described below.

Morris water maze test

Spatial learning and memory were evaluated at 12 weeks after OPC transplantation using the Morris water maze. An open circular pool [100 cm (diameter) $\times$ 50 cm (height)] was filled with water ($24 \pm 2^\circ\text{C}$), and the black platform [6 cm (diameter) $\times$ 28 cm (height)] was submerged 2–3 cm below the water surface at a depth of 30 cm. For the navigation trial, rats underwent four trials daily with different starting points over 5 consecutive days and twice daily. Rats escaping the platform were restricted within 60 s and allowed to rest on the platform for 10 s. The space-probe trial with the platform removed took place on day 6 over 60 s. The escape latency, number of platform crossings, number of platform quadrant crossings, and time spent on the platform quadrant were recorded.

Adhesive-removal test

The adhesive-removal test was used as a sensitive method for detecting and monitoring sensorimotor deficits after CNS injury. Before each test, the animals were placed in an experimental home cage for 2 min for acclimation. After testing, each rat was sterilized with alcohol. Prior to formal testing, animals

were trained for 3 days to familiarize them with the test and exclude any outliers from subsequent testing. The longest detection time for each rat was 180 s. When the sticker was not removed after 180 s, the time was recorded as 180 s. The rats were gently scuffed and inverted, with a small quarter-circle adhesive placed on either the left or right forepaw. The time of contact and removal of the dot was measured separately, with the adhesive placed on alternating forepaws. The test was repeated three times for both the right and left forepaws.

Cylinder test

To evaluate forelimb-use asymmetry resulting from ipsilateral brain damage, the ratio of contralateral forelimb use was analyzed at 12 weeks post-transplantation. Each animal was individually placed in a glass cylinder [20 cm (diameter) × 40 cm (height)], and forelimb use was observed during the exploratory behavior of the rats for 5 min. The evaluation process was blind, and all mice were adaptively trained before starting the experiment. During the experiment, the environment was kept quiet to avoid unnecessary stimulation. Contact between the weight-bearing forepaw and cylinder wall during a full rear was recorded as right (normal), left (impaired), or both. Left forelimb preference was calculated as $[(\text{impaired forepaw} + 1/2\text{both})/(\text{normal forepaw} + \text{impaired forepaw} + \text{both}) \times 100\%]$.

Gait analysis

Gait analysis was performed at 12 weeks post-PWMI using an automated computer-assisted method (Xin Hai Hua Yi Instrument Co., Beijing, China), and data were collected and analyzed using Gait Analysis Lab software (v.5.0). An enclosed glass walkway was illuminated from the long edge with a green light that was completely internally reflected. The light reflected by the paws as they touched the glass floor was captured and transformed into a digital image. Food pellets were placed in the goal box as a motivator cue for a successful run. Training and experimental procedures were performed under the same conditions. Training started 1 week before surgery and lasted for at least 3 days until the animals learned to cross the walkway without interruption. An observer blinded to the experimental groups performed behavioral and data analyses. The data were then blindly assessed by trained technicians.

Statistical analysis

Data are presented as the mean ± standard deviation and mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism software (v.5.0; GraphPad, Inc. San Diego, CA, USA). The results of the water maze space-exploration experiment were evaluated by two-way analysis of variance, followed by multiple comparisons. Other results were analyzed by one-way analysis of variance. A $p < 0.05$ was considered to indicate significant results.

Results

Cell identification

Immunofluorescence staining of hOPC-specific markers revealed elevated expression of A2B5, NG2, PDGFR- α , SOX10, Olig2, and O1 but low expression of GFAP and Tuj-1 (Fig. 1). hOPCs labeled with CM-Dil showed red fluorescence, with a labeling rate of > 95% (Fig. 2).

Detection of transplanted hOPCs after intranasal delivery

hOPCs were located throughout the brain at 24 h after intranasal administration (Fig. 3). DAPI and CM-Dil double-positive cells were detected throughout the layers of the olfactory bulb, cerebral cortex, hippocampus, and corpus callosum and cells were detected around the lateral ventricle in rats. These data demonstrate that hOPCs reached the damaged area after HI.

OPC transplantation stimulates myelin regeneration

Myelin regeneration after intranasal transplantation in the brain was investigated using two methods. First, we investigated MBP expression via immunohistochemistry. Microscope images indicated a decrease in the fluorescent area in the cingulate and corpus callosum. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to measure and calculate the ratio of the MBP-positive area of the injured side cingulate and corpus callosum to the total area (Fig. 4). Compared with the sham group, the rate of the MBP-positive area on the injured side of the PWMI group was significantly reduced ($p < 0.01$) from $38.39 \pm 2.49\%$ (sham) to $26.47 \pm 2.49\%$ (PWMI) in the cingulate and from $29.92 \pm 3.52\%$ (sham) to $20.97 \pm 1.88\%$ (PWMI) in the corpus callosum. Compared with the PWMI group, the rate of the MBP-positive area in the nasal group was significantly increased ($p < 0.01$) from $26.47 \pm 2.49\%$ (PWMI) to $33.22 \pm 4.61\%$ (nasal group) in the cingulate and from $20.97 \pm 1.88\%$ (PWMI) to $28.10 \pm 3.622\%$ (nasal group) in the corpus callosum.

To further verify the promotion of myelin regeneration by hOPCs, the corpus callosum was evaluated by electron microscopy following selection of representative micrographs of axons in the genu of the corpus callosum. The shape of the myelin sheath in the sham group was normal, with a uniform appearance, clear boundaries, and no loose separation and/or disintegration of the lamellar structure. The myelin sheath of the model group was loose, separated, and disintegrated and the lamellar structure was loose. Moreover, separation and disintegration of the myelin sheath in the nasal-transplantation group was rare, and the proportion of normal fragments of the dense layer in the concentric circle structure of the myelin sheath was larger than that of the model group. Compared with the sham group, the average g-ratio and myelin-sheath-damage scores in the PWMI group were significantly increased ($p < 0.001$), indicating reductions in myelin-sheath thickness and a serious degree of damage. Compared with the PWMI group, the average g-ratio and myelin-sheath-damage scores in the transplantation group were significantly reduced ($p < 0.001$ and $p < 0.05$, respectively), suggesting an increase in myelin-sheath thickness and restoration of the degree of myelin-sheath injury (Fig. 5).

Improvement in neurological function after HI

The Morris water maze test was performed to evaluate long-term spatial-learning and memory ability. In the acquisition trial, rats in the PWMI group showed decreased spatial-learning ability and significantly increased escape latency ($p < 0.01$ vs. sham) on training days. In contrast, rats in the hOPC nasal-transplantation group showed better learning ability and a remarkable reduction in escape latency on training days relative to in the PWMI group ($p < 0.05$ for days 1 and 2; $p < 0.01$ for days 3–5). There was no significant difference between groups in swimming time and distance (data not shown). In the retention trial, no significant differences in the percentage of time spent in the target quadrant and frequency across the platform were observed between the PWMI and hOPC nasal-transplantation groups. Compared with the sham group, the number of platform quadrant crossings was reduced in the PWMI group ($p < 0.01$), although this number increased in the nasal-transplantation group ($p < 0.01$). Representative swimming pathways of the different groups on days 5 and 6 in the water maze test are shown in Fig. 6A and 6B.

To compare functional outcomes after HI injury, rats were subjected to the adhesive-removal test at 12 weeks after cell transplantation to evaluate their sensorimotor functional deficits (Fig. 6C). White matter damage in the right hemisphere impaired sensory and motor function of the left forelimb of rats, as indicated by the increased time required to detect and remove the adhesive as compared with that observed on the contralateral side (right forelimb). Compared with the sham group, the time to contact the left forelimb sticker in the PWMI group increased from 3.69 ± 2.11 to 10.84 ± 4.88 s ($p < 0.01$) and the time to remove the sticker increased from 4.63 ± 2.97 to 10.14 ± 4.43 s ($p < 0.01$). Compared with the PWMI group, the time to contact the left forelimb sticker in the nasal-transplantation group was reduced from 10.84 ± 4.88 to 5.92 ± 2.26 s ($p < 0.01$) and the time to remove the sticker was reduced from 10.14 ± 4.43 to 6.16 ± 1.79 s ($p > 0.05$), showing a trend of improvement in the symptoms of neurological impairment.

In the cylinder test assesses forelimb asymmetry, the frequency of left forelimb use in the PWMI group was significantly decreased ($p < 0.05$) relative to that in the sham group at the end of the examination (Fig. 6D). No significant difference in forelimb use was observed between the PWMI and nasal-transplantation groups ($p = 0.531$).

To analyze motor coordination, gait analysis was performed at 12 weeks after intranasal delivery (Fig. 7). Compared with the sham group, the stride length decreased in the left forelimb and right hindlimb in the PWMI group ($p < 0.05$); compared with the PWMI group, stride length increased in the left forelimb ($p < 0.05$) and right hindlimb ($p > 0.05$) in the nasal-transplantation group. Additionally, the walking cycle and contralateral coordination of the left forelimb increased after intranasal delivery. Indicators of walking posture, such as standard deviation of body angle ($p < 0.05$), absolute mean body angle ($p < 0.01$), and standard deviation of absolute body angle ($p < 0.01$), were increased in ischemic–anoxic rats relative to in the sham group. Furthermore, the three indicators of walking posture decreased after nasal transplantation.

Discussion

WMI in premature infants occurs mainly because of hypoperfusion of the brain and intrauterine infection in the mother. Given the enhanced response mediated by glutamate receptors and lack of effective antioxidant protection, cells of the early oligodendrocyte lineage are susceptible to hypoperfusion and subsequent activation of microglia and astrocytes [12–15]. Currently, there is no specific treatment for WMI in premature infants.

Stem cell-based therapies are emerging as novel and highly promising strategies for various CNS diseases, including ischemic stroke, hemorrhagic stroke, and spinal cord injury [16–18]. Stem cell therapy may be useful for treating WMI in premature infants. Following arterial transplantation, cells can reach the lesions directly and quickly; however, microthrombus formation, the complicated operation, and other high risks are limitations of this approach [19, 20]. After vein transplantation, the cell migration time is long and mobility rate is low, and cells can easily migrate to other organs, including the liver and lungs, potentially leading to pulmonary embolism [21]. Additionally, stereotactic brain transplantation causes mechanical damage to the brain tissue, with increased fluid volume following transplantation potentially capable of squeezing surrounding tissues and producing a volume-occupying effect, which can cause further brain damage [22]. Moreover, lumbar puncture and transplantation are demanding and difficult operations that can easily cause inflammation [23]. Stem cells transplanted from the lateral ventricle can be distributed with the cerebrospinal fluid to the entire brain and spinal cord, thereby making this a suitable method for treating extensive lesions but unsuitable for small-scale lesions. These invasive transplantation methods are unsuitable for clinical implementation in premature infants because of their immature organs, immature respiratory centers, poor cardiac function, and low immune function. Therefore, it is necessary to explore non-invasive, convenient, and repetitive transplantation pathways involving minimal clinical trauma.

Premature infants have short nasal passages, a thin nasal mucosal barrier, and abundant submucosal blood vessels, and studies have shown that cells transplanted through the nose can efficiently enter damaged areas [24]. Therefore, nasal transplantation may represent a non-invasive method for repairing brain damage.

Neural stem cells, mesenchymal stem cells, and olfactory sheath cells have been widely used in the treatment of brain injury; however, the ability of these cells to produce OLs (the main types of damaged cells in brain WMI) is limited, as the rate of differentiation into OLs and myelin formation *in vivo* is very low. Thus, it is difficult for these stem cells to form a myelin sheath in the CNS and initiate cell replacement following transplantation into the brain.

OPCs are precursors of OLs (the main cells forming the myelin sheath). The myelin sheath not only conducts bioelectric signals but also provides nutritional support for axons, with continued loss of myelin causing axonal loss that leads to motor and sensory dysfunction [25]. Increasing evidence has shown that the proliferation, migration, and secretion of upregulated nutritional factors from OPCs can promote neuroprotection and long-term repair of diseased tissues, with OPCs as the best seed cells for transplantation therapy [26]. Following OPC transplantation into a mouse model of neonatal

metachromatic leukodystrophy, the mice survived to adulthood with no tumors, along with survival of myelinated and endogenous OLs in the white matter of adult metachromatic leukodystrophy mice. Moreover, the transplanted cells reduced sulfide accumulation in the CNS and prevented electrophysiological and motor deficits in untreated metachromatic leukodystrophy mice [27]. Other studies using a WMI model reported that OPCs play a neuroprotective role by promoting endogenous neural stem cell proliferation in the injured inferior lateral ventricle and near the dentate gyrus, thereby inhibiting neuronal apoptosis and stimulating brain-derived neurotrophic factor and B-cell lymphoma-2 expression [28]. OPCs can migrate to damaged areas and integrate into the host brain tissue, where they further proliferate and differentiate to form mature OLs and envelope axons, repair damaged white matter, and play a role in cell replacement [29]. Additionally, OPCs can interact with vascular endothelial cells of the blood-brain barrier to reduce the permeability and protect the integrity of this barrier.

To the best of our knowledge, hOPCs have not been evaluated for the treatment of PWMI in rats via nasal transplantation. Detection of hOPC-specific markers revealed elevated expression of characteristic markers of OLs, including A2B5, NG2, SOX10, PDGFR, Olig2 and O1, with low expression of GFAP (representing astrocytes) and Tuj-1 (representing neurons), indicating that the induced cells were high-purity OPCs. Here, 1×10^6 hOPCs were transplanted through the nasal route 3 days after injury, with results indicating that the fluorescence-labeled cells survived and migrated to the damaged cortex, corpus callosum, hippocampus, and surrounding lateral ventricles within 3 days after transplantation. The cells may have migrated through the olfactory nerve pathway into the brain [30]. To evaluate the effect of transnasal transplantation of hOPCs, we assessed nerve-repair function via immunofluorescence staining, TEM, and behavioral experiments at 12 weeks after transplantation. The results showed that compared with the PWMI group, hOPC transplantation significantly promoted myelin-sheath regeneration and alleviated nerve-function injury. These results suggest that transnasal transplantation represents a new strategy for treating PWMI.

We selected the time-frame for nasal transplantation as 3 days after injury to observe cell migration mainly based on the following considerations: 1) at 3–7 days after ischemic–hypoxic brain injury, various biochemical substances and cytokines in the injured area begin to become activated, and various chemicals released during the acute phase of WMI exert a chemotactic effect conducive to the migration and homing of heterotopic stem cells around the lesion [31]; and 2) rodent studies showed that the size of the injured area no longer increases within 4 days after neonatal HI [32]. Therefore, the time-frame for this neuroprotective strategy involves preventing cell death and inflammation within 4 days after injury. Transplanted stem cells can alleviate the inflammatory response and induce apoptosis during the acute phase of brain injury. Additionally, the time-frame of nasal transplantation was selected as 8 days after injury to allow observation of the long-term effect of cell transplantation mainly based on the following considerations: the microenvironment formed following injury of necrotic brain tissue in the surrounding area and dislocated red blood cells, as well as caused the release of a large amount of excitatory neurotransmitters, free radicals, and inflammatory factors during the acute phase, which can directly threaten the survival, proliferation, and differentiation of transplanted cells. Stem cells transplanted 8

days after injury were chosen to avoid peak inflammation, reduce stem cell death, and replace dead cells in the brain tissue during the recovery period, thereby allowing the formation of new neural circuits and improve the symptoms of neurological deficits.

Conclusion

Our results showed that transnasal transplantation of hOPCs significantly promoted remyelination and improved learning, memory, and cognitive deficits following HI, as well as improved sensorimotor function. These findings suggest that hOPCs can be used as a clinical treatment strategy for PWMI and provide insight into a novel transplantation method for treating WMI using stem cells in premature infants.

Abbreviations

WMI: white matter injury

Pre-OLs: pre-oligodendrocytes

CNS: central nervous system

PWMI: premature white matter injury

OPC: oligodendrocyte precursor cell

A2B5: ganglioside

NG2: neural/glial antigen 2

PDGFR- α : PDGF receptor- α

SOX10: SRY-box transcription factor 10

Olig2: oligodendrocyte transcription factor 2

GFAP: glial fibrillary acidic protein

Tuj-1: class III β -tubulin

HI: hypoxia–ischemia

MBP: myelin basic protein

TEM: transmission electron microscopy

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Contributions

ZJ and YYX planned and performed the experiments and completed the manuscript. GQ and WXH revised the manuscript. ZX, WZY, WQ, HY, YD and ZHP helped ZJ to perform experiments. WYJ and GAS supervised the study. ZJ, YYX, and LZ designed the study. The authors discussed and approved the manuscript.

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Ethics declarations

Ethics approval and consent to participate

All experimental procedures were approved by the ethics committee of the Sixth Medical Center of Chinese People's Liberation Army General Hospital (Approval Number:SCXK-2012-0001).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Immunofluorescence staining of hOPC-specific markers. Expression of A2B5, NG2, PDGFR- α , SOX10, Olig2, and O1 was elevated, whereas GFAP and Tuj-1 expression was low. (A–H) Expression levels of A2B5, NG2, PDGFR- α , SOX10, Olig2, O1, GFAP, and Tuj-1, respectively. Scale bar = 100 μ m.

Figure 2

CM-Dil labeling of hOPCs in vitro. Scale bars for (A) 200 μ m, (B) 100 μ m, (C) 50 μ m.

Figure 3

Tracking transplanted cells in the brain after intranasal delivery. Animals were euthanized at 3 days post-transplantation (P9), and hOPCs were pre-labeled with CM-Dil (red) for 4 h prior to transplantation. DAPI (blue) staining was used to identify nuclei in both the host cells and hOPCs. Cells were found in the (A) olfactory bulb, (B) cortex, (C) hippocampus, (D) corpus callosum, and (E) lateral ventricle. Scale bar = 100 μm . (F) Representative images of transplanted cells above the lateral ventricle. Scale bar = 50 μm .

Figure 4

Intranasally-transplanted hOPCs stimulate myelin regeneration after injury. (A, B) Representative micrographs of MBP in the cingulate and corpus callosum in the sham, PWMI, and nasal-transplantation groups at 3 months post-transplantation. Scale bar = 200 μ m. (C) MBP immunofluorescence showing comparable staining in the cingulum, corpus callosum, and capsula externa between rats treated with hOPCs and those left untreated.

Figure 5

Electron micrographs of myelinated axons in each group. (A) Representative electron micrographs of myelinated axons in the corpus callosum in the sham, PWMI, and nasal-transplantation groups at 3-months post-transplantation. Scale bar = 5 μm . (B) High-magnification (60,000 $\times$) images are shown. Scale bar = 500 nm. (C) Graphs showing g-ratios in the three groups (n = 100 axons/rat; n = 3 rats/group. *p < 0.05, **p < 0.01, ***p < 0.001). (D) Scatter plots and linear regression of the axon diameter vs. g-ratio from myelinated fibers. Data represent the mean $\pm$ standard error of the mean. (E) Scoring of damage to axonal ultrastructure in the three groups. Bars represent the mean $\pm$ standard deviation. (F) Schematic of the measured parameters.

Figure 6

Transplanted hOPCs ameliorate impairments in learning, memory, cognition, and sensorimotor function after HI. (A) Performance of the Morris water maze test. In the acquisition trial, PWMI rats showed significantly longer latency than rats in the sham and nasal-transplantation groups. In the retention trial, the number of platform quadrant crossings was reduced in PWMI rats relative to rats in the sham and nasal-transplantation groups. The percentage of time spent in the target quadrant and frequency across

the platform showed no significance difference between the PWMI and nasal-transplantation groups. (B) Representative swimming pathways of different groups on days 5 and 6 in the water maze test. Performance of the (C) adhesive-removal test and (D) cylinder test in all groups. * $p < 0.05$, ** $p < 0.01$ vs. sham; # $p < 0.05$, ## $p < 0.01$ vs. PWMI.

Figure 7

Gait analysis 12 weeks after intranasal delivery to analyze motor coordination. (A) Left forelimb stride was shorter in PWMI rats relative to that in rats of the sham group; however, nasal-transplantation rats showed a longer stride than rats in the PWMI group. (B, C) Compared with the PWMI group, the walking cycle and contralateral coordination of the left forelimb were increased in the nasal-transplantation group. (D, E) Indicators of walking posture, such as standard deviation of body angle ($p < 0.05$), absolute mean body angle ($p < 0.01$), and standard deviation of absolute body angle ($p < 0.01$), were increased in ischemic–anoxic rats relative to those in the sham group, whereas these parameters were decreased in the nasal-transplantation group.