Study on the Safety of Human Oligodendrocyte Precursor Cells Transplantation in Young Animals and Its Efficacy on Myelination

Haipeng Zhou
Southern Medical University  https://orcid.org/0000-0002-2240-5362

Siliang Lu
Sixth Medical Center of PLA General Hospital

Ke Li
Sixth Medical Center of PLA General Hospital

Yinxiang Yang
Sixth Medical Center of PLA General Hospital

Caiyan Hu
Sixth Medical Center of PLA General Hospital

Zhaoyan Wang
Sixth Medical Center of PLA General Hospital

Qian Wang
Sixth Medical Center of PLA General Hospital

Ying He
Sixth Medical Center of PLA General Hospital

Xiaohua Wang
Sixth Medical Center of PLA General Hospital

Dou Ye
Sixth Medical Center of PLA General Hospital

Qian Guan
Sixth Medical Center of PLA General Hospital

Jing Zang
Sixth Medical Center of PLA General Hospital

Chang Liu
Sixth Medical Center of PLA General Hospital

Suqing Qu
Sixth Medical Center of PLA General Hospital

Zuo Luan (nfykdxxzb@163.com)
Southern Medical University
Abstract

Oligodendrocyte precursor cells (OPCs), which can differentiate into myelinating oligodendrocytes during embryonic development, are an important potential source for myelin repair or regeneration. To date, OPCs from human sources (hOPCs) remain limited. In this study, we aimed to evaluate the safety and remyelination capacity of hOPCs developed in our laboratory by transplanting them into the lateral ventricles of Sprague–Dawley rats of different ages. The toxicity, biodistribution, and tumor formation abilities of the injected hOPCs were examined by evaluating rats’ vital signs, developmental indicators, neural reflexes, along with hematological, immunological, and pathological assessments. In addition, the hOPCs were transplanted into the corpus callosum of shiverer mice to verify cell myelination efficacy. Overall, our results showed that transplanted hOPCs into young mice showed no toxicity against their organ function or immune system, engrafted only in the brain, and caused no tissue proliferation or tumor formation. In terms of efficacy, the transplanted hOPCs formed myelin in the corpus callosum, alleviated the trembling phenotype of shiverer mice, and promoted normal development. The transplantation of hOPCs is safe and can effectively form myelin in the brain, thereby providing a theoretical basis for the future clinical transplantation of hOPCs.

Introduction

Newborns are one of the most at-risk groups for developing demyelinating diseases (Pakpoor et al. 2018), which are characterized by the loss or damage of the central nervous system’s oligodendrocytes (OLs), the primary myelin sheath-forming cells in the brain and spinal cord. Myelin replacement through normal proliferation and differentiation of OLs is an effective treatment strategy (Chen et al. 2013; Georgiou et al. 2017; Mozafari et al. 2020; Novak et al. 2017). Previous studies have evaluated cell transplantation as a potential treatment strategy of demyelinating diseases. Since stem cells have diverse properties, including nerve regeneration, neuroprotection, and neurotrophic (Marina et al. 2018; Ribeiro et al. 2015; Shrestha et al. 2014), stem cell transplantation is considered one of the most promising treatment strategies for demyelinating diseases. OLs populations can be replenished from various stem cell sources, such as neural, embryonic, and induced pluripotent stem cells (Czepiel et al. 2015). These stem cell sources must reach an oligodendrocyte precursor cells (OPCs) state during differentiation into oligodendrocytes (Sim et al. 2006), which have greater proliferation and migration ability than OLs (Medved et al. 2020); therefore, OPCs are more suitable for post-transplant survival than OLs, which would also increase the extent of myelination.

Safety and efficacy of cell transplantation for the treatment of neonatal demyelinating diseases is critical. Newborns may also show different adverse reactions compared to adults, after cell transplantation (Lu et al. 2015). Safety studies on stem cell transplantation for other neurological diseases have been reported with various cell types. For example, bone marrow mesenchymal stem cell transplantation for spinal cord injury (SCI) (Geffner et al. 2008); human allogeneic central nervous system stem cell transplantation for Pelizaeus-Merzbacher disease (Gupta et al. 2019); human neural stem cells...
transplantation for Alzheimer's disease (Mcginley et al. 2018). These studies have confirmed the safety of stem cells in the treatment of neurological diseases, providing a reference for our research.

Currently, most transplanted cells were of rat or mouse origin (Nishiyama et al. 2009; Santos et al. 2019; Stolt et al. 2006; Zhou et al. 2000), and only a limited studies have used human-derived OPCs (hOPCs) (Hu et al. 2009; Namchaiw et al. 2019; Wang et al. 2013b). In this study, we transplanted OPCs of human origin. In a previous study, isolated neural stem cells (hNSCs) from human fetal brain tissue have been used, which were then differentiated into hOPCs (Lin et al. 2020). This human-derived OPCs have good proliferation ability and can be stably expanded to the fifth generation. More importantly, it only takes 6 to 7 days to be induced from hNSCs, saving precious time for clinical transplantation. According to reports, it takes at least 20 days for OPCs induced by other cells (Douvaras et al. 2014; Izrael et al. 2007; Keirstead et al. 2005; Wang et al. 2013a). However, cell transplantation safety warrants further investigations. Although we have previously demonstrated the remyelination ability of our developed hOPCs (Wu et al. 2017), in this study, the remyelination ability of hOPCs will be further evaluated.

In this study, we have focused on hOPCs generated from human fetal brain NSC and assessed their toxicity following transplantation into the lateral ventricle of young animals and its efficacy on myelination.

**Materials And Methods**

1. **Cultivation and identification of hOPCs**

hOPCs were prepared by the Pediatric Laboratory of the Sixth Medical Center of the Chinese People's Liberation Army General Hospital using previously established methods for their cultivation and identification (Text S1). The study was not pre-registered at the start of the methods. Next, the hOPCs were identified by flow cytometry and cell morphology. hOPCs were surface-stained with PDGFR-α BV421 mouse anti-human (Cat. #562799, BD Biosciences, Franklin Lake, New Jersey, USA), A2B5 PE mouse anti-human (Cat. #130-093-581, Miltenyi Biotec, Bergisch-Gladbach, Germany), and NG2 APC mouse anti-human (Cat. #FAB2585A, R&D Systems, Minnesota, USA) antibodies for flow cytometry (FACSanto II, BD Biosciences, Franklin Lake, New Jersey, USA).

2. **Animals**

Sprague–Dawley (SD) rats and homozygous shiverer mice (The Jackson Laboratory, Maine) were maintained in a specific pathogen-free environment at the Sixth Medical Center of PLA General Hospital. The room temperature was set to 23 ± 2°C, the humidity to 60 ± 10%, with light and dark cycles of 12 h. Animals had *ad libitum* access to sterile food and water. In this study, 48 newborn SD rats were randomly selected from a pool of 60, and 24 newborn shiverer mice were randomly selected from a pool of 40. For the inclusion criteria, the difference between the weight of each animal and the average weight was set to < 1g, whereas otherwise excluded; no animals were excluded since all fit the inclusion criteria. The total number of one-day-old rats or mice in each the experimental and control groups was 12 (6 males and 6
females), and each cage contained four animals. During animal testing following transplantation, the
tester was unaware of the animal grouping. Table S1 shows the initial weight of the animals at one day
old. We estimated the sample size based on the initial weight of the animal using the following equation:

\[ N = \frac{(Z_{\alpha} + Z_{\beta})^2 \times 2\sigma^2}{\delta^2} \]

\[ \delta = \frac{(S_1 + S_2)^2}{2} \]

In the formula: \( \alpha = 0.05 \), \( \beta = 0.9 \), \( Z_{0.05} = 1.96 \), \( Z_{0.9} = 1.28 \), \( \sigma = \frac{X_1 - X_2}{2} \), \( \delta = X_1 - X_2 \). \( \alpha \) refers to Type I
error while \( \beta \) to Type II, and \( S_1 \) and \( S_2 \) refer to the standard deviation of the animals' weight, while \( Z_1 \) and
\( Z_2 \) refer to the mean.

3. Safety of hOPCs transplantation into the lateral ventricle of SD rats

In the acute toxicity experiments, the transplantation was performed in the rats on post-partum day 6. We
measured weight, tibia length, righting reflex, and cliff avoidance reaction every 2 days over the 14 days
following transplantation (21 days after birth). These tests were conducted at 6 pm on the day of the
experiment. On day 14, the young rats were sacrificed by cervical dislocation. In the chronic toxicity
experiment, a total of three transplants were performed, and the transplantation time points corresponded
to post-partum days 6, 20, and 40. Following the initial transplantation, the body weight and tibial length
of the mice were measured every 3 days until the end of the experiment (post-partum day 90). These tests
were conducted at 6 pm on the day of the experiment. After the third transplantation, blood was taken
from the tail vein every 2 weeks for routine and biochemical blood tests. The haematology test was
divided into two days: The first day was the transplantation group, while the second day was the control.
The daily experiment duration was from 10 am to 5 pm. After the third blood collection, blood immune
factor detection, and blood immune cell detection were carried out simultaneously. Immunofluorescence
was used to detect the residual hOPCs in the various tissues. Before being sacrificed, all transplanted rats
were photographed. After sacrifice, the coefficients of the heart, liver, spleen, lung, kidney, brain, thymus,
spinal cord, testes, and ovary organs were calculated using the formula \( OC = m / M \% \), whereby \( m \)
represents the organ's weight, and \( M \) represents the rat's weight.

3.1 Transplantation of hOPCs into the lateral ventricle of SD rats

HOPCs were transplanted into the right lateral ventricle of the experimental group (n = 12), and saline was
injected into the right lateral ventricle of the control group (n = 12). The subgroups were the following: 1)

HOPCs were transplanted into the right lateral ventricle of the experimental group (n = 12), and saline was
injected into the right lateral ventricle of the control group (n = 12). The subgroups were the following: 1)

male cell (MC, n = 6), 2) female cell (FC, n = 6), 3) male saline (MS, n = 6), and 4) female saline (FS, n =
6). Animals were anaesthetised with isoflurane anaesthesia (3-4%) immediately prior to surgery to
minimise animal suffering. The animal's head was then placed into a stereotaxic apparatus mask
(Stoelting, USA), and the concentration of isoflurane was maintained at 2–2.5%. The anesthetized with
isoflurane rats were fixed on a rat brain stereotaxic apparatus (Stoelting, USA) and their heads were
disinfected before the skin was incised to expose the skull. The anterior fontanelle was the zero point. The injection site (anterior and posterior, midline-lateral, and depth) was approached based on the zero point (Windrem et al. 2004). In the acute toxicity experiments, the lateral ventricle of each rat was injected with either 5 μL of hOPCs (1×10^6 cells) or 5 μL of saline; injection rate: 0.5 μL/min; the lateral ventricle coordinates were (AP: 0.5 mm, ML: 1 mm, DV: 2.0 mm). In the chronic toxicity experiments, each rat was transplanted three times; once every 2 weeks. On the first, fourth, and seventh week each rat was injected a volume of 5 μL, 10 μL, and 10 μL, respectively, of either hOPCs (1×10^6 cells, 2×10^6 cells, and 2×10^6 cells) or saline. The coordinates of the lateral ventricle corresponding to the three transplants were (AP: 0.5 mm, ML: 1 mm, DV: 2.0 mm), (AP: 0.5 mm, ML: 1 mm, DV: 1.0 mm), and (AP: 0.5 mm, ML: 1 mm, DV: 1.0 mm). After transplantation, the incision was sutured, the skin was disinfected, and the young rats were returned to their mothers for feeding and were closely observed for 48 h. After weaning, the female and male rats were reared in separate cages.

### 3.2 Neural reflex detection

As part of the acute toxicity experiments, the neural reflex test was performed. Righting reflex: After transplantation, whether the supine young rats could turn over and touch the ground within 2 s was observed and assessed. If all young rats were positive for three consecutive tests on the same day, the day was considered a standard day. Cliff avoidance reflex: Starting from the first day after transplantation, young rats were placed on the edge of a workbench with a height of 30 cm. If the rats retreated or turned around from the edge within 90 s, the reaction was considered positive. If all rats in the same cage were positive, the day was considered a standard day.

### 3.3 Blood routine and serum biochemistry

We collected three blood samples after the final transplantation in the chronic toxicity experiment at 2-week intervals (weeks 9, 11, and 13, after birth). The blood was transferred to an anticoagulant tube containing EDTA. The XT2000iv blood analyzer (SYSMEX, JAPAN) was used to determine the blood routine. Detection indicators included red blood cells, white blood cells, platelets, hematocrit, hemoglobin, and mean platelet volume. Approximately 1 mL of blood was centrifuged at 2,000 rpm for 10 minutes at 4 °C, and the serum was obtained for further biochemical testing. Using a biochemical analyzer (HITACHI 7020, JAPAN), we measured alanine aminotransferase, aspartate aminotransferase, albumin, creatinine, urea, glucose, total protein, total cholesterol, and triglycerides.

### 3.4 Detection of cytokines in peripheral blood

At the last blood collection in the chronic toxicity experiment (week 13 after birth), whole blood samples were placed at room temperature for 2 h, before being centrifuged at 2,000 rpm for 20 min at 4 °C, and the supernatant was collected for testing. An enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minnesota, USA) was used to detect peripheral blood immune factors. Detection indicators included IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-6. The assay was performed according to the manufacturer’s instructions.
3.5 Detection of peripheral blood immune cells

In the final blood collection of the chronic toxicity experiment (week 13 after birth), the fresh blood sample was mixed with an equivalent volume of normal saline and slowly added to the test tube containing lymphocyte separation solution (Cat. # abs930, Absin Bioscience Inc, Shanghai, China). Then the blood was centrifuged at 2,000 rpm for 20 min at 4 °C to be divided into its compartments: plasma, WBCs, and RBCs (top to bottom). A capillary tube was used to collect the WBC-rich cell suspension above the RBC layer, and this was transferred to another test tube. Hanks solution was added without Ca\(^{2+}\) and Mg\(^{2+}\) ions; it was then mixed and centrifuged at 2,000 rpm for 10 min at 4 °C. Finally, a cell suspension was made with RPMI 1640 medium. Cell suspensions were surface-stained with CD3 APC mouse anti-human (Cat. #562799, BD Biosciences, Franklin Lake, New Jersey, USA), CD4 APC-A750 (Cat. #130-093-581, Miltenyi Biotec, Bergisch-Gladbach, Germany), CD8 PC5.5 (Cat. #FAB2585A, R&D Systems, Minnesota, USA), and CD45RA PC7 mouse anti-human antibodies for flow cytometry (FACSanto II, BD Biosciences, Franklin Lake, New Jersey, USA).

3.6 Detection of spleen immune cells

Following the final blood collection in the chronic toxicity experiments (week 13 after birth), rats were sacrificed by cervical dislocation. The spleen was removed under aseptic conditions and rinsed three times with normal saline. It was then cut and ground into a homogenate without tissue mass, filtered with a 40 μm cell sieve (Cat. #352340, BD Biosciences, Franklin Lake, New Jersey, USA), and diluted with saline to prepare a spleen cell suspension. The cell suspension was added to the centrifuge tube containing lymphocyte separation solution and centrifuged at 2,000 rpm for 20 min. A capillary tube was used to collect the WBC-rich cell suspension over the RBC layer, which was then transferred to a 50 mL centrifuge tube and mixed with physiological saline. Thereafter, this underwent centrifugation at 1,700 rpm for 7 min. Finally, an RPMI 1640 medium was used to prepare a cell suspension. Cell suspensions were then surface-stained with CD3 APC, CD4 APC-A750, and CD8 PC5.5 mouse anti-human antibodies for flow cytometry.

3.7 Histopathological analysis and biodistribution of transplanted hOPCs

After the chronic toxicity experiments (week 13 after birth), the main organs (heart, liver, spleen, kidney, thymus, spinal cord, testes, ovary, brain, and lung) were fixed in 10% formalin. The tissue sections were stained with hematoxylin and eosin, and an upright optical microscope (NIKON ECLIPSE CI, JAPAN) was used for observation, inspection, and evaluation of histopathological lesions. Immunofluorescence staining with STEM121 (mouse anti-human STEM121 antibody, Cat. # Y40410, 1:500, Takara Bio, Japan) was performed to label the residual hOPCs in each tissue.

4. Study of the effectiveness of hOPCs-induced myelination

4.1 hOPCs transplantation in shiverer mice and tremor assessment
Newborn shiverer mice were transplanted within one day after birth. Animals were anaesthetised as described in section 3.1 at an isoflurane induction concentration of 2-3%, and a maintenance concentration of 1-1.5%. A mouse brain stereotaxic apparatus (Stoelting, USA) was used to inject hOPCs (1.5 μL; 2x10^5 cells) into the right corpus callosum of shiverer mice (n = 6). The transplant coordinates were as follows: AP, 0.5 mm; ML, 1 mm; and DV, 0.5 mm. The control group consisted of six randomly selected non-transplanted homozygotes. After the operation, the body weights were recorded at the same time every 7 days until the end of the experiment. At 30, 60, and 90 days after transplantation, the tremor was assessed. The indicators included the amplitude of the tremor and the duration of continuous tremor.

4.2 Electron microscopy and tissue immunofluorescence staining

Three months after transplantation, mouse brain samples were imaged using a transmission electron microscope (TEM, H7650-B, HITACHI, Tokyo, Japan). A field of view was randomly selected for each mouse, and the G-ratio was calculated (myelin inner diameter / myelin outer diameter) according to the amount of myelin in the field of view. The remaining mouse brain tissues were immunostained with myelin basic protein (MBP) (rat anti-MBP antibody, Cat. #Ab7349, Abcam, Cambridge, UK). A fluorescence microscope (IX-70, Olympus Corporation) was used to image the samples and the average optical density of MBP was calculated using Image Pro Plus 6.0 (Media Cybernetics).

5. Statistical analysis

Statistical analyses were conducted using SPSS version 22.0. Data expressed as the mean ± standard error. The normality of the data was evaluated using a normal distribution graph. The mean of continuous data was analyzed using an analysis of variance. Two-way ANOVA was used to compare the groups. Values of p < 0.05 were considered indicative of statistically significant differences.

Results

1. hOPCs are identified by three biomarkers: PDGFR-α, A2B5, and NG2

The hOPCs were identified based on their morphology and quantified of biomarkers using flow cytometry. Brightfield microscopy verified that hOPCs have a typical bipolar, bead-like morphology (Fig.1a) and can be stably passaged up to the fifth generation. Flow cytometry analyses showed that the proportion of PDGFR-α+ cells was 72.27 ± 3.01%. A2B5+ cells accounted for 25.87 ± 3.02%, and NG2+ cells accounted for 18.38 ± 1.51% (Fig. 1b) of the total cell population.

2. Safety of hOPCs transplantation into the lateral ventricle of SD rats

2.1 Evaluation of growth and neural reflex

The body surface characteristics of both hOPCs transplanted and control groups were normal. The heart, liver, spleen, lung, kidney, brain, thymus, spinal cord, testes, ovary, and other major organs were normal in
appearance, and displayed no signs of inflammation, exudation, or bleeding (Fig. 2a). The organ coefficient results showed no statistically significant difference between the hOPCs transplanted and the control groups (Fig. 2b). The results of the tibia length test showed no significant difference between the hOPCs transplanted and control groups (acute: p = 0.9881, chronic: p = 0.9078; Fig. 3a). The weight measurement results showed no significant difference between the hOPCs transplanted and control groups (acute: p = 0.6409, chronic: p = 0.7815); however, the weight difference between females and males in the chronic toxicity experiment was statistically significant (p = 0.0479, Fig. 3b). In the acute toxicity experiment, the righting reflex evaluation results showed that the number of days rats needed to reach a standard day was 14.66 ± 0.88 days for the hOPCs transplanted group and 14.25 ± 1.06 days for the control group; however, the difference between the two groups was not statistically significant (p = 0.3066). The cliff avoidance reflex evaluation results showed that the number of days required for rats to achieve a standard response was 14.92 ± 0.67 days for the hOPCs transplanted group and 14.83 ± 0.94 days for the control group, which was not statistically significant (p = 0.8044; Fig. 3c).

2.2 Hematology and immunological assessments

For the chronic toxicity test, routine blood examination and peripheral blood biochemical results showed no significant difference between the hOPCs transplanted and the control group (Fig. 4, Table S2-S3). Analyses for serum cytokines and immune cell detection in the peripheral blood and in the spleen showed no significant difference between the two groups (Fig. 5a–e, Table S4).

2.3 Transplantation, cell labeling, and histopathological evaluation

STEM121 immunofluorescence staining of the main organ tissues showed that human-derived cells were found only in the brain tissue (Fig. 6a). In the chronic toxicity test, histopathological evaluations of the rat’s main organs (heart, liver, kidney, thymus, spinal cord, testes, ovary, brain, and lung) showed no signs of inflammation (Fig. 6b).

3. Confirmation of hOPCs myelination

Three months post hOPCs transplantation, brain imaging with transmission electron microscopy showed a small amount of mature myelin in the homozygous non-transplanted shiverer mice of the control group. Most of the myelin sheaths did not form dense lines, and the overall structure was loose. In the hOPCs transplanted group, the number of mature myelin sheaths was large, and dense lines were formed (Fig. 7a). The results of the myelin G-ratio analysis showed that the hOPCs transplanted and the control group values were 63.59 ± 3.36% and 94.42 ± 1.42%, respectively, which was statistically significant (p < 0.0001, Fig. 7b). MBP immunostaining confirmed myelination in the corpus callosum (Fig. 7c). The mean optical density values, derived from the MBP labeling, were 0.071 ± 0.005 and 0.017 ± 0.002 for the hOPCs transplanted and the control group, respectively, which was statistically significant (p < 0.0001, Fig. 7d). The body mass index test results showed a statistically significant difference in body weight gain between the two groups, within 91 days post-transplantation (p = 0.0029, Fig. 7e). The observation results of the tremor amplitude and the duration of a single continuous tremor showed that the difference
between the cell transplantation group and control group, within 91 days post-transplantation, was statistically significant (Fig. 7f-g, Table S5).

**Discussion**

In this study, we transplanted different doses of human-derived OPCs into the lateral ventricles of SD rats of different ages and assessed their growth and development along with their nervous system and showed that hOPCs transplantation had no toxic side effects. In this study, the biodistribution of the transplanted hOPCs was limited to the brain and resulted in no tissue proliferation or tumor formation. In addition, we also confirmed that hOPCs transplanted into the corpus callosum of Shiverer mice can form myelin, thereby alleviating the shivering behavior caused by demyelination, and promoting the normal growth of these mice.

Safety studies of stem cell transplantation consider the following parameters: The purity and stability of the stem cell line, dose and route of transplantation, species and age of the experimental animals, experimental period, and the selection of test indicators (Hoberman and Lewis 2012). In this study, considering the developmental characteristics of newborns and animal species, we began transplanting hOPCs into rats of different ages and performed both acute short-term (21 days) and chronic long-term (90 days) toxicity assessments. In addition, various indicators were evaluated at different time points in these rats for dynamic monitoring.

We first assessed the purity and stability of the hOPCs through a cell morphology and biomarker analysis. hOPCs are typical bipolar bead-like cells expressing PDGFR-α, A2B5, NG2, SOX10, and Olig2 (Marques et al. 2018), which is consistent with our results. Subsequently, three aspects related to stem cell safety, namely toxicity, biodistribution, and tumorigenesis, were examined. Herein, the acute toxicity experiment within 21 days of cell transplantation, showed no transplant-associated deaths among the rats. Further, growth indicators, such as skin color, abdominal hair, body weight, and bone development, along with breathing, exercise, digestion, and nervous system related indicators were all normal. In the chronic toxicity test, to avoid complications such as infection, hematoma, and brain herniation caused by multiple transplants, the transplantation was conducted every 2 weeks with a slow injection speed rate of 0.5 μL/min. Hematological indicators and immunological tests from multiple time points, and the levels of relevant indicators of routine blood tests were all normal. Although WBC and PLT fluctuated in the three tests, no symptoms of infection or anemia were found. Liver and kidney function and blood lipids were normal, as were the GLU levels; the levels of immune-related factors and immune cells in the blood and spleen were also within the normal range. H&E staining of major organs showed normal structure with no inflammation. Regarding hOPCs biodistribution after lateral ventricle transplantation, STM121 immunofluorescence staining of major peripheral organs showed that the transplanted hOPCs only engrafted in the brain tissues and did not localize in other regions (at 90 days). In another study of OPCs transplantation for the treatment of spinal cord injury, the migration distance of OPCs in the spinal cord parenchyma was only approximately 3 cm (Manley et al. 2017). Therefore, hOPCs transplanted into the lateral ventricle would not be expected to migrate to other organs. Moreover, since hOPCs have several
stem cell characteristics, the risk of tumor formation after transplantation remains a possibility (Norenberg et al. 2004; Wang et al. 2015). In this study, even on week 13 of the chronic toxicity experiment, no neoplasms were observed on the macroscopic and microscopic aspects of SD rats in the transplantation group; moreover, the weight change and organ coefficient of the transplantation group were not different from those of the control group. In addition, a pathological analysis showed no tissue proliferation or tumor formation in any of the major peripheral organs.

Limited studies have previously assessed the myelination potential of transplanted human-derived OPCs. hOPCs can migrate to demyelinating sites or the region of myelin injury after transplantation, and further differentiate into oligodendrocytes, forming myelin sheaths around axons, and activating host endogenous progenitor cells to improve the remyelination of axons (Farhangi et al. 2019). To minimize the influence of host endogenous progenitor cells, we used the Shiverer mice to evaluate the myelinating ability of hOPCs. The Shiverer mouse is an animal model with congenital myelination loss suitable for testing the myelinating ability of transplanted cells (Windrem et al. 2008). Consistent with previous findings, transmission electron microscopy and MBP immunofluorescence staining conducted 90 days after hOPCs transplantation confirmed the myelination of the corpus callosum of the Shiverer mice. Further, the tremor phenotype was relieved in the transplanted group. The appetite of the transplanted mice also increased, resulting in greater body weight than the control group mice.

Together, these data can provide guidance for future clinical trials of lateral ventricle transplantation of hOPCs for the treatment of demyelinating diseases. First, the safe dose of hOPCs for single transplantation of cells in 1-week-old newborn SD rats is \(1 \times 10^6\) with an injection volume of 5 µL. At post-partum week 7, the safe dose of single transplanted cells can be increased to \(2 \times 10^6\) with an injection volume of 10 µL can be used. The efficacious number of myelinating cells to alleviate the tremor phenotype in newborn Shiverer mice is \(2 \times 10^5\) with an injection volume of 3 µL. These experimental doses will not cause brain herniation, and the cell suspension will not overflow. Therefore, we recommend that the experimental dose in future preclinical tests should not greatly exceed this dose. Second, the detection indicators after transplantation in this study may provide references for clinical trials. The selection of observation indicators significantly influences the experimental study of juvenile animal toxicity. According to published guidelines, general evaluation indicators usually include gross observation, vital signs, growth and development, hematology, pathology, and gross observation. If a specific target organ or system is considered, it can be combined with reproductive function, sexual development function, immune function, and central nervous system function indicators (Duarte et al. 2020; Silva-Lima et al. 2010). In addition, the length of the observation period, transplantation time point, and the index detection time point of this study can be used as reference values for future clinical trials. In this study, the observation period for the acute toxicity experiment lasted 21 days after birth, and the observation period for the chronic toxicity experiment lasted 90 days. The transplantation time points consisted of the first, fourth, and seventh weeks post-partum, while time points for the detection of hematological indicators were 9 weeks, 11 weeks, and 13 weeks post-partum. Studies have shown that within 22 days of birth, rats are equivalent to human infants/toddlers (< 2 years), in which basic
physiological development characteristics have been established. Moreover, within 46 days of birth, rats are equivalent to human children at 2–12 years of age. Important organs, such as the heart, lungs, and liver, are typically fully developed in mice at 13 weeks of age, which is equivalent to the adolescent period (12-16 years old) of humans. The development of neural reflexes, learning and memory, kidneys, and other systems are relatively complete when rats are approximately 20 weeks old, which is equivalent to human adulthood (Baldrick 2004; Smialowicz 2002).

Conclusion

In conclusion, this study provided evidence of the safety of human-derived OPCs transplantation in young animals, and confirmed the efficacy of its myelination potential. Together, these results provide insights into the safety and effectiveness of hOPCs for future clinical studies for the treatment of neonatal myelin diseases.

Declarations

ETHICAL STATEMENT:

Ethics approval and consent to participate

The preparation of the hOPCs was approved by the Scientific Council and Ethics Committee of The Sixth Medical Center of PLA General Hospital (Application No. 2015013). All animal experiments in this study were performed according to protocols approved by the Sixth Medical Center of the PLA General Hospital Animal Care and Use Committee (Application No. SCXK-2012-0001). Inform consent from all the women who donated their aborted foetuses was obtained.

Consent for publication

All the authors of this study agree to participate and publication.

Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors report no conflicts of interest in this work.

Funding

This research was supported by the National Key R&D Program of China (NO.2017YFA0104200).

Authors’ Contributions
HPZ, SQQ, and ZL designed the experiments. HPZ, SLL and KL performed cell transplantation and other animal experiment operations experiments. HPZ, YH, ZYW, QW and CYH prepared the cells to be transplanted. HPZ, SLL and KL analyzed the data. YXY, XHW, DY, QG, JZ, and CL contributed materials/reagents/analysis tools. HPZ wrote the manuscript.

Acknowledgements

The authors appreciate the technical support and other help from Leping Zhang, Jing Zang, Chang Liu, and Qian Guan. We would like to thank Editage (www.editage.cn) for English language editing.

References

Baldrick P (2004) Developing drugs for pediatric use: a role for juvenile animal studies? Regulatory toxicology and pharmacology : RTP 39:381-389 doi:10.1016/j.yrtph.2004.03.004

Chen Y, Yi Q, Liu G, Shen X, Xuan L, Tian Y (2013) Cerebral white matter injury and damage to myelin sheath following whole-brain ischemia Brain research 1495:11-17 doi:10.1016/j.brainres.2012.12.006

Czepiel M, Boddeke E, Copray S (2015) Human oligodendrocytes in remyelination research Glia 63:513-530 doi:10.1002/glia.22769

Douvaras P et al. (2014) Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells Stem cell reports 3:250-259 doi:10.1016/j.stemcr.2014.06.012

Duarte D, Beatriz da Silva Lima M, Sepodes B (2020) The translational value of animal models in orphan medicines designations for rare paediatric neurological diseases Regulatory toxicology and pharmacology : RTP 118:104810 doi:10.1016/j.yrtph.2020.104810

Farhangi S, Dehghan S, Totonchi M, Javan MJMS, Disorders R (2019) In vivo conversion of astrocytes to oligodendrocyte lineage cells in adult mice demyelinated brains by Sox2 Mult Scler Relat Disord 28:263-272

Geffner LF et al. (2008) 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 17:1277-1293

Georgiou E et al. (2017) Gene therapy targeting oligodendrocytes provides therapeutic benefit in a leukodystrophy model Brain : a journal of neurology 140:599-616 doi:10.1093/brain/aww351

Gupta N et al. (2019) Long-Term Safety, Immunologic Response, and Imaging Outcomes following Neural Stem Cell Transplantation for Pelizaeus-Merzbacher Disease Stem Cell Reports 13:254-261 doi:10.1016/j.stemcr.2019.07.002
Hoberman AM, Lewis EM (2012) Juvenile Toxicity Study Design for the Rodent and Rabbit. John Wiley & Sons, Inc.,

Hu BY, Du ZW, Li XJ, Ayala M, Zhang SC (2009) Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects Development 136:1443-1452 doi:10.1242/dev.029447

Izrael M et al. (2007) Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo Molecular and cellular neurosciences 34:310-323 doi:10.1016/j.mcn.2006.11.008

Keirstead H, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, Steward O (2005) Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury The Journal of neuroscience : the official journal of the Society for Neuroscience 25:4694-4705 doi:10.1523/jneurosci.0311-05.2005

Lin L, Amissah E, Gamble G, Crowther C, Harding J (2020) Impact of macronutrient supplements on later growth of children born preterm or small for gestational age: A systematic review and meta-analysis of randomised and quasirandomised controlled trials PLoS medicine 17:e1003122 doi:10.1371/journal.pmed.1003122

Lu Y, Yang Y, Wang Z, Wang C, Du Q, Wang Q, Luan Z (2015) Isolation and culture of human oligodendrocyte precursor cells from neurospheres Brain research bulletin 118:17-24 doi:10.1016/j.brainresbull.2015.08.008

Manley NC, Priest CA, Denham J, Wirth ED, Lebkowski JSJSCTM (2017) Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cells: Preclinical Efficacy and Safety in Cervical Spinal Cord Injury Stem Cells Transl Med 10:6

Marina G, Vladislav V, Harrell C, Crissy F, Nemanja J, Nebojsa A, Miodrag SJIJoMS (2018) Stem Cells Therapy for Spinal Cord Injury 19:1039

Marques S et al. (2018) Transcriptional Convergence of Oligodendrocyte Lineage Progenitors during Development Developmental cell 46:504-517.e507 doi:10.1016/j.devcel.2018.07.005

Mcginley LM et al. (2018) Human neural stem cell transplantation improves cognition in a murine model of Alzheimer's disease Scientific Reports 8:14776

Medved J et al. (2020) Novel guanidine compounds inhibit platelet-derived growth factor receptor alpha transcription and oligodendrocyte precursor cell proliferation Glia doi:10.1002/glia.23930

Mozafari S et al. (2020) Multiple sclerosis iPS-derived oligodendroglia conserve their properties to functionally interact with axons and glia in vivo Science advances 6 doi:10.1126/sciadv.abc6983
Namchaiw P, Wen H, Mayrhofer F, Chechneva O, Biswas S, Deng W (2019) Temporal and partial inhibition of GLI1 in neural stem cells (NSCs) results in the early maturation of NSC derived oligodendrocytes in vitro Stem Cell Res Ther 10:272 doi:10.1186/s13287-019-1374-y

Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity Nat Rev Neurosci 10:9-22 doi:10.1038/nrn2495

Norenberg MD, Smith J, Marcillo AJJoN (2004) The pathology of human spinal cord injury: defining the problems J Neurotrauma 21:429

Novak I et al. (2017) Early, Accurate Diagnosis and Early Intervention in Cerebral Palsy: Advances in Diagnosis and Treatment JAMA pediatrics 171:897-907 doi:10.1001/jamapediatrics.2017.1689

Pakpoor J, Goldacre R, Schmierer K, Giovannoni G, Waubant E, Goldacre MJMs (2018) Psychiatric disorders in children with demyelinating diseases of the central nervous system Multiple Sclerosis Journal 24:1243-1250 doi:10.1177/1352458517719150

Ribeiro TB et al. (2015) Neuroprotection and immunomodulation by xenografted human mesenchymal stem cells following spinal cord ventral root avulsion Scientific Reports 5:16167

Santos AK, Vieira MS, Vasconcellos R, Goulart VAM, Kihara AH, Resende RR (2019) Decoding cell signalling and regulation of oligodendrocyte differentiation Semin Cell Dev Biol 95:54-73 doi:10.1016/j.semcdb.2018.05.020

Shrestha B, Coykendall K, Li Y, Moon A, Priyadarshani P, Yao LJScr, therapy (2014) Repair of injured spinal cord using biomaterial scaffolds and stem cells Stem Cell Res Ther 5:91 doi:10.1186/scrt480

Silva-Lima B, Due Theilade-Thomsen M, Carleer J, Vidal J, Tomasi P, Saint-Raymond A (2010) Juvenile animal studies for the development of paediatric medicines: a description and conclusions from a European Medicines Agency workshop on juvenile animal testing for nonclinical assessors Birth defects research Part B, Developmental and reproductive toxicology 89:467-473 doi:10.1002/bdrb.20257

Sim FJ et al. (2006) Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation Ann Neurol 59:763-779 doi:10.1002/ana.20812

Smialowicz R (2002) The rat as a model in developmental immunotoxicology Human & experimental toxicology 21:513-519 doi:10.1191/0960327102ht290oa

Stolt CC et al. (2006) SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function Dev Cell 11:697-709 doi:10.1016/j.devcel.2006.08.011

Wang F, Qi HX, Zu Z, Mishra A, Tang C, Gore JC, Chen LMJMRiM (2015) Multiparametric MRI reveals dynamic changes in molecular signatures of injured spinal cord in monkeys Magn Reson Med 74:1125-
Wang S et al. (2013a) Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination Cell stem cell 12:252-264 doi:10.1016/j.stem.2012.12.002

Wang S et al. (2013b) Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination Cell Stem Cell 12:252-264 doi:10.1016/j.stem.2012.12.002

Windrem M et al. (2008) Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse Cell Stem Cell 2:26

Windrem MS et al. (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain Nat Med 10:93-97 doi:10.1038/nm974

Wu C, Wang Z, Yang Y, Luan ZJzddekzzCjocp (2017) [Long-term effect of oligodendrocyte precursor cell transplantation on a rat model of white matter injury in the preterm infant] Zhongguo Dang Dai Er Ke Za Zhi 19:1003-1007

Zhou Q, Wang S, Anderson DJ (2000) Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors Neuron 25:331-343 doi:10.1016/s0896-6273(00)80898-3