GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Curative effect and safety of intrathecal transplantation of neural stem cells for the treatment of cerebral hemorrhage

Y.Z. Xue, X.X. Li, L. Li, S.L. Pang, J.G. Yao and P.L. Hao

Department of Neurology, Yishui Central Hospital, ShanDong, China

Corresponding author: Y.Z. Xue
E-mail: yzplcn@yeah.net

Genet. Mol. Res. 13 (4): 8294-8300 (2014)
Received June 4, 2013
Accepted August 27, 2014
Published October 20, 2014
DOI http://dx.doi.org/10.4238/2014.October.20.5

ABSTRACT. In this study, we aimed to explore the curative effect and safety of neural stem cell intrathecal transplantation for the treatment of cerebral hemorrhage. We transplanted 4.0 x 10^8 neural stem cells per procedure into the subarachnoid space by lumbar puncture 7 days after cerebral hemorrhage, twice a week, a total of 4 times. NIHSS scores and brain CT scans were conducted to assess neural functions and the volume of perihematoma lesions in patients on days 1, 7, 14, 21, and 28. We found that the NIHSS scores and the volume of the perihematoma lesions were significantly reduced after day 14. The differences before and after treatment were highly significant in intra- and between-group comparisons ($P < 0.05$). There were no adverse reactions, except for transient fever and shivering in a few patients. Our data suggest that the use of neural stem cells in intrathecal transplantation for the treatment of cerebral hemorrhage is safe and effective.

Key words: Cerebral hemorrhage; Neural stem cell transplantation; Curative effect; Adverse reaction; Lumbar puncture

INTRODUCTION

Intracerebral hemorrhage (ICH) causes severe neurological deficits and a large morbidity rate in patients. This can be attributed to neuronal apoptosis and severe injury of glial cells due to the mechanical compression of the hematoma, as well as the secondary damage caused by cerebral edema and local inflammation (Kanvacki et al., 2006; Xi et al., 2004, 2006). Since medical therapy against ICH, such as mechanical removal of hematoma, prevention of edema formation by drugs, and reduction of intracranial pressure has limited effectiveness, alternative approaches such as stem cell-based cell therapy are required (Gebel and Broderick, 2000; NINDS ICH Workshop Participants, 2005).

Recent progress in stem cell biology has opened up new approaches to therapeutic strategies to replace the lost neural cells by transplantation of neural stem cells (NSCs) in CNS injury and disease (McKay, 1997; Flax et al., 1998; Gage, 2000; Kim, 2004; Lindvall and Kokaia, 2006; Muller et al., 2006). Stem cells are initial cells with abilities for self-renewal (Darsalia et al., 2007), high proliferation, and multiple-directional differentiation, which can relieve dysfunctional neurons with certain growth factors and give rise to neurons, astrocytes, and oligodendrocytes to replace the injured cells (Aboody et al., 2000; Bjorklund and Lindvall, 2000; Modo et al., 2002; Savitz et al., 2002). Previous studies indicated that NSCs engrafted in animal models of ICH survive and ameliorate neurological deficits in the animals (Jeong et al., 2003; Lee et al., 2007; Lee et al., 2008). Jeong et al. (2003) reported that intravenously transplanted NSCs could enter the rat brain with ICH, undergo migration, and thus improve functional recovery.

From October 2010 to March 2012, 20 cerebral hemorrhage patients received NSCs intrathecal transplantation in our department. By evaluating the curative effects and the side effects, we expected to open a new way for the treatment of cerebral hemorrhage.

MATERIAL AND METHODS

General information

Forty cases of acute hypertensive ICH patients were divided randomly into 2 groups: 20 patients in the treatment group received routine treatment and neural stem cell transplantation and 20 patients in the control group received routine treatment. There were 13 males and 7 females, aged 40 to 70 years, with an average age of 58.1 ± 8.6 years in the treatment group; and 11 males and 9 females, aged 41 to 69 years, with an average age of 60.3 ± 8.7 years in the control group.

The inclusion criteria were basal ganglia hemorrhage, bleeding amount between 30 to 50 mL, age between 30 and 75 years, an onset time within 24 h, myodynamia below or at 3 levels, and medical history of the patient's family.

The exclusion criteria were hemorrhage after cerebral infarction, hemorrhage secondary to other diseases (such as hemopathy, cancer, etc.), severe hepatic or renal dysfunction, patients with atrial fibrillation or severe cardiac dysfunction, complicated severe gastrointestinal bleeding or severe infection, and disagreement of the patient's family for transplantation.

Treatment methods

The 2 groups received identical basic treatments such as blood pressure control, anti-exudative, and activation of neuronal metabolism. In addition, patients in the treatment group received NSC transplantation therapy. About 4.0 x 10^8 NSCs were transplanted into the subarachnoid space by lumbar puncture 7 days after cerebral hemorrhage, twice per week, a total of 4 times. The NSCs came from the Red Cross Intervention Hospital Stem Cells Center, Shandong Province.

Observed indexes

Clinical therapeutic efficacy

NIHSS scores were conducted at day 1, 7, 14, 21, and 28 after hemorrhage.

Imaging indexes

The brain CT scan was conducted to assess patients' neural functions and the volume of perihematoma lesions. The volume of hematoma and the lesion around the hematoma were calculated by Duo Tian formula. The amount of bleeding equaled to $\pi/6$ multiplied by the maximum length of hematoma, the maximum width of hematoma, and the number of layers. This method had been validated by plane measurement (Kothari et al., 1996). The volume of the lesion around the hematoma equaled the volume of the hematoma plus the volume of the low-density lesions around hematoma subtracted from the actual volume of the hematoma. The measurements were done in milliliters. The study endpoint was the day 28 after hemorrhage.

Adverse reactions

Adverse reactions such as fever, chills, dizziness, and increased blood pressure were observed and recorded in detail after NSC transplantation.

Statistical analysis

All data was expressed as means ± standard deviation (means ± SD) and analyzed with the statistical software SPSS13.0 software. The difference was detected by Student *t*-test. The difference of categorical data was detected by chi-square test. P values < 0.05 was considered to be statistically significant.

RESULTS

Baseline data for the 2 groups (treatment and control)

Information about the 2 groups, such as gender, age, systolic blood pressure, diastolic blood pressure, glucose, bleeding volume, and NIHSS scores of the first day, followed a normal distribution. There was no significant difference between the 2 groups by Student *t*-test and chi-square test (P > 0.05) (Table 1).

Table 1. Baseline conditions of the two groups.

	Control group	Treatment group	t/χ^2 value	P value
Male/female	11/9	13/7	$\chi^2 = 0.417$	0.519
Age	59.05 ± 6.93	58.25 ± 7.24	t = -0.357	0.723
Systolic pressure (mmHg)	176.90 ± 7.90	171.50 ± 11.11	t = -1.772	0.085
Diastolic pressure (mmHg)	103.65 ± 10.86	108.25 ± 8.12	t = 1.517	0.138
Blood Glucose (mM)	10.39 ± 1.30	9.39 ± 2.34	t = -1.66	0.106
Hemorrhagic dose (mL)	42.90 ± 3.55	44.30 ± 3.04	t = 1.340	0.189
Lesion volume around hematoma (mL)	15.43 ± 2.44	17.39 ± 5.23	t = 1.519	0.137
NIHSS scores	19.26 ± 3.57	18.56 ± 5.69	t = 0.965	0.278

Comparison of neural function defect (NIHSS) between the 2 groups (treatment and control) before and after treatment

The NIHSS scores of the 2 groups before treatment followed a normal distribution. No statistical difference was observed by the Student *t*-test (P > 0.05). The NIHSS scores of the 2 groups met the "sphericity symmetric" assumption at the same time after onset. The differences in the NIHSS scores were statistically significant by the variance analysis except for day 7. The NIHSS scores of treatment group declined compared with the control group (Figure 1, Table 2).

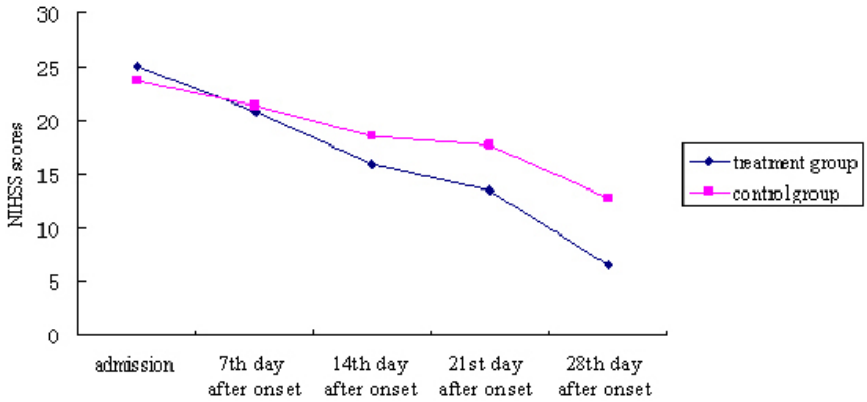

Figure 1. NIHSS scores on admission and different time points after onset.

Table 2. NIHSS scores on admission and different time points after onset (means ± SD).

	Control group	Treatment group
Admission	23.70 ± 3.55	25.12 ± 3.58
7th day after onset	21.43 ± 2.93	20.90 ± 3.55$^{\Delta}$
14th day after onset	18.50 ± 3.55	15.93 ± 3.50$^{\triangledown}$
21st day after onset	17.75 ± 2.96	13.45 ± 1.77*
28th day after onset	12.81 ± 2.38	6.50 ± 1.18*

$^{\Delta}$Differences of the NIHSS scores between the two groups 7th day after onset were no statistically significant, P > 0.05; $^{\triangledown}$Differences of the NIHSS scores between the two groups 14th day after onset were considered to be statistically significant, P < 0.05; *Differences of the NIHSS scores between the two groups 21st and 28th days after onset were considered to be statistically significant, P < 0.01.

Volume around the hematoma lesions of the 2 groups (treatment and control) at different time points before and after treatment

The volumes around the hematoma lesion showed no statistical differences between the 2 groups at admission and day 7 after onset ($P > 0.05$). The volumes around the hematoma lesions obviously decreased from the beginning of day 14 after onset, and the most obvious change was at day 28. Both groups showed parallel decline, but the decline in the treatment group was highly obvious. Irrespective of whether the comparison was between the 2 groups or a comparison of the same group before and after treatment, the differences were obviously significant ($P < 0.01$) (Figure 2, Table 3).

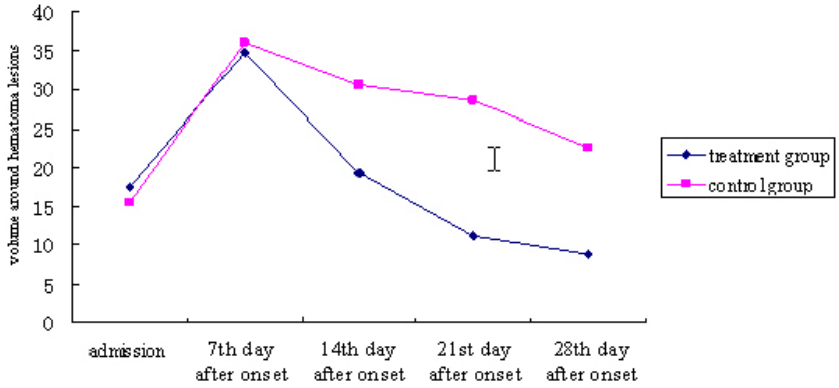

Figure 2. Volume around the hematoma lesions between the two groups.

Table 3. Volume around the hematoma lesions on admission and different time points after onset (mL).

	Control group	Treatment group
Admission	15.43 ± 2.44	17.39 ± 5.23
7th day after onset	36.3 ± 6.23	34.8 ± 7.10[Δ]
14th day after onset	30.62 ± 4.76	19.25 ± 6.51*
21st day after onset	28.61 ± 4.73	11.14 ± 1.76*
28th day after onset	22.6 ± 4.73	9.79 ± 5.34*

[Δ]Differences of the volume around the hematoma lesions between the two groups 7th day after onset were no statistically significant, $P > 0.05$; *Differences of volume around the hematoma lesions between the two groups 14th, 21st, and 28th days after onset were considered to be statistically significant, $P < 0.01$.

Adverse reactions

Two patients had transient chill and fever, and the body temperature reached 38°C during the treatment. These phenomena happened into 24 h after transplantation, which could be alleviated after intravenous injection of 10 mg dexamethasone. The dizziness or stretching, incidentally, did not need special treatment.

DISCUSSION

Traditional treatment of ICH includes chemotherapy and surgery. The therapeutic purpose was to remove the hematoma and reduce the secondary damage caused by the hematoma-

induced oppression on the surrounding brain tissue. However, 50% of patients still survived with severe disability (Zhang et al., 2006). The repair of damaged neurons with respect to structure and function became the hotspot of research on the treatment of cerebral hemorrhage.

In our study, exogenous NSCs were transplanted to the subarachnoid region by lumbar puncture. The neural function greatly improved since the second transplantation. By the time of study termination, the NIHSS scores reached 6.50 ± 1.18, and they were found to be obviously lower than the values of the control group and those observed before treatment. There was significant statistical difference. The volumes of damaged brain tissue around the hematoma had obviously decreased. By the time of study termination, it reached 9.79 ± 5.34 mL and was found to be obviously lower than that in the control group. The intrathecal transplantation of NSCs for the treatment of cerebral hemorrhage was found to be effective from the clinical manifestations and imaging studies, the findings that were in agreement with previous research (Lee et al., 2008). This indirectly showed that the lack of nerve function in patients with cerebral hemorrhage was connected with the damage of neural tissue around the hematoma, and therefore, it was very important to promote the recovery of neurological function by timely repair of the damaged neural tissue.

On one hand, the transplanted cells migrate to the damaged area under the guidance of chemotactic factors (Lee et al., 2008) and then secrete a variety of nerve growth factors and neurotrophic factors (Nagai et al., 2007), which could inhibit neuronal apoptosis, promote the regeneration of blood vessels and axons (Lapergue et al., 2007), improve the microenvironment of the damaged area, and protect the organization around the hematoma. On the other hand, the transplanted cells can activate the proliferation and differentiation of the endogenous NSCs (Masuda et al., 2007), replace the damaged neurons, and rebuild neural circuits. Improvement in the symptoms cannot be attributed to the transformation of transplanted cells into nerve cells (Castro et al., 2002); instead, it can be due to the autocrine function of some factors that play a role in the protection of damaged cells. To some extent, this effect stopped the progress of cell apoptosis.

Although we did not use immunosuppressive agents, we did not observe immune rejection before and after transplantation. This was due to the low immunogenicity of the NSCs and the "immune immunity" of the brain to the NSCs. A few patients had transient fever and shiver within 24 h of transplantation; this phenomenon alleviated after symptomatic treatment. The dizziness or stretching incidentally did not need special treatment. Therefore, intrathecal transplantation of NSCs for the treatment of cerebral hemorrhage was safe and effective.

In most experimental research and clinical treatment studies, 18F-Fluorodopa method was used to evaluate the curative effects of stem cell transplantation. However, our hospital lacks this infrastructure, and therefore, we were unable to monitor the state and function of hRPE *in situ* after transplantation in the brain.

REFERENCES

Aboody KS, Brown A, Rainov NG, Bower KA, et al. (2000). Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc. Natl. Acad. Sci. U. S. A.* 97: 12846-12851.

Bjorklund A and Lindvall O (2000). Cell replacement therapies for central nervous system disorders. *Nat. Neurosci.* 3: 537-544.

Castro RF, Jackson KA, Goodell MA, Robertson CS, et al. (2002). Failure of bone marrow cells to transdifferentiate into neural cells *in vivo*. *Science* 297: 1299.

Darsalia V, Kallur T and Kokaia Z (2007). Survival, migration and neuronal differentiation of human fetal striatal and

cortical neural stem cells grafted in stroke-damaged rat striatum. *Eur. J. Neurosci.* 26: 605-614.
Flax JD, Aurora S, Yang C, Simonin C, et al. (1998). Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. *Nat. Biotechnol.* 16: 1033-1039.
Gage FH (2000). Mammalian neural stem cells. *Science* 287: 1433-1438.
Gebel JM and Broderick JP (2000). Intracerebral hemorrhage. *Neurol. Clin.* 18: 419-438.
Jeong SW, Chu K, Jung KH, Kim SU, et al. (2003). Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. *Stroke* 34: 2258-2263.
Kanvacki Z, Kowianski P, Witko-wska M, Karwacka M, et al. (2006). The pathophysiology of intracerebral haemorrhage. *Folia Morphol.* 65: 295-300.
Kim SU (2004). Human neural stem cells genetically modified for brain repair in neurological disorders. *Neuropathology* 24: 159-171.
Kothari RU, Brott T, Broderick JP, Barsan WG, et al. (1996). The ABCs of measuring intracerebral hemorrhage volumes. *Stroke* 27: 1304-1305.
Lapergue B, Mohammad A and Shuaib A (2007). Endothelial progenitor cells and cerebrovascular diseases. *Prog. Neurobiol.* 83: 349-362.
Lee HJ, Kim KS, Kim EJ, Choi HB, et al. (2007). Brain transplantation of immortalized human neural stem cells promotes functional recovery in mouse intracerebral hemorrhage stroke model. *Stem Cells* 25: 1204-1212.
Lee ST, Chu K, Jung KH, Kim SJ, et al. (2008). Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain* 131: 616-629.
Lindvall O and Kokaia Z (2006). Stem cells for the treatment of neurological disorders. *Nature* 441: 1094-1096.
Masuda T, Isobe Y, Aihara N, Furuyama F, et al. (2007). Increase in neurogenesis and neuroblast migration after a small intracerebral hemorrhage in rats. *Neurosci. Lett.* 425: 114-119.
McKay R (1997). Stem cells in the central nervous system. *Science* 276: 66-71.
Modo M, Stroemer RP, Tang E, Patel S, et al. (2002). Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. *Stroke* 33: 2270-2278.
Muller FJ, Snyder EY and Loring JF (2006). Gene therapy: can neural stem cells deliver? *Nat. Rev. Neurosci.* 7: 75-84.
Nagai A, Kim WK, Lee HJ, Jeong HS, et al. (2007). Multilineage potential of stable human mesenchymal stem cell line derived from fetal marrow. *PLoS One* 2: e1272.
NINDS ICH Workshop Participants (2005). Priorities for clinical research in intracerebral hemorrhage: report from a National Institute of Neurological Disorders and Stroke workshop. *Stroke* 36: 23-41.
Savitz SI, Rosenbaum DM, Dinsmore JH, Wechsler LR, et al. (2002). Cell transplantation for stroke. *Ann. Neurol.* 52: 266-275.
Xi G, Fewel ME, Hua Y, Thompson BG Jr, et al. (2004). Intracerebral hemorrhage: pathophysiology and therapy. *Neurocrit. Care* 1: 5-18.
Xi G, Keep RF and Hoff JT (2006). Mechanisms of brain injury after intracerebral haemorrhage. *Lancet Neurol.* 5: 53-63.
Zhang H, Huang Z, Xu Y and Zhang S (2006). Differentiation and neurological benefit of the mesenchymal stem cells transplanted into the rat brain following intracerebral hemorrhage. *Neurol. Res.* 28: 104-112.