



## Therapeutic Potential of Umbilical Cord Blood Stem Cells on Brain Damage of a Model of Stroke

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### ABSTRACT

**Introduction:** Human cord blood-derived stem cells are a rich source of stem cells as well as precursors. With regard to the researchers have focused on the therapeutic potential of stem cell in the neurological disease such as stroke, the aim of this study was the investigation of the therapeutic effects of human cord blood-derived stem cells in cerebral ischemia on rat. **Methods:** This study was carried out on young rats. Firstly, to create a laboratory model of ischemic stroke, carotid artery of animals was occluded for 30 minutes. Then, umbilical cord blood cells were isolated and labeled using bromodeoxyuridine and  $2 \times 10^5$  cells were injected into the experimental group via the tail vein. Rats with hypoxic conditions were used as a sham group. A group of animals did not receive any injection or surgeries were used as a control. **Results:** Obtained results were evaluated based on behavioral responses and immunohistochemistry, with emphasis on areas of putamen and caudate nucleus in the control, sham and experimental groups. Our results indicated that behavioral recovery was observed in the experimental group compared to the either the sham or the control group. However, histological studies demonstrated a low percent of tissue injury in the experimental group in comparison with the sham group. **Conclusions:** Stem cell transplantation is beneficial for the brain tissue reparation after hypoxic ischemic cell death.

### Introduction

Nowadays, stroke continues to be the third leading cause of death and a main factor of disability in the community. About half of all strokes cause by ligation of cerebral artery by a blood clot that so-called cerebral thrombosis. Other major causes of stroke are embolism or a hemorrhage. If the blood supply to a part of the brain disrupted or interrupted, this area of the brain will be unable to function. This situation commonly called a stroke. Early treatment after a stroke is administration of anti-thrombolytic drugs to dissolve blood clots within artery. Although a rehabilitation treatment such as physiotherapy and speech therapy is essential to improve the movement after stroke, however the complete improvement is unusual.<sup>1</sup> Thus, for treatment of this neurological disorder, the medical community is in need for a definitive treatment. Many studies were focused on the therapeutic effects of stem cells in damages of body tissues particularly in the nervous system. Cell therapy can be considered as a main treatment for stroke. Stem cells are un-

differentiated cells that maintain the ability of proliferation and production of their precursor cells in response to stimulation of specific cell types in the body. Our knowledge about these cells is rapidly increasing and recently the new landscape of restorative strategies is found in brain disorders such as ischemic stroke, Parkinson, brain injury, Huntington's, amyotrophic sclerosis, multiple sclerosis and Alzheimer diseases.<sup>2-5</sup> It is hoped that progression in using stem cell transplantation in the brain damage caused to be practical.

Types of stem cells produce during development of mammals from different sources that may be useful in treatment of brain ischemia. These cells include embryonic stem cells<sup>6</sup>, neural stem cells<sup>7</sup>, stem cells derived from bone marrow, cord blood and adipose tissue.<sup>8</sup> Umbilical cord blood contains a population of stem cells that have the potential to become nerve cells. Furthermore, these cells are less immunologic than bone marrow-derived mesenchymal cells. Hence, this study was designed to investigate motor recovery and replacing of the

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stem cells derived from umbilical cord blood in ischemic tissue in the experimental models of ischemic stroke using immunohistochemistry and behavioral tests.

## Materials and methods

### *The collection of cord blood and isolation of mononuclear cells and labeling them with BrdU*

Umbilical cord blood of mothers aged 20 to 40 years who had no history of smoking, alcohol or typical disease were collected in special bags containing dextrose adenine citrate phosphate. Blood samples were rapidly diluted 1:1 in buffer (PBS without calcium and magnesium) and then diluted to a ratio of 8:3 in 15 ml centrifuge tubes with Ficoll-Paque centrifugation at 800 g for 20 min at room temperature. Then the supernatant was carefully removed using a pipette and transferred into new tubes. After washing twice with PBS, samples were centrifuged at 800 g for 10 to 20 minutes. Collected cells were suspended at the bottom of the tube with 1 ml of serum. After that, the mononuclear cells were transferred into the culture flasks containing RPMI medium enriched with 10% bovine serum and antibiotics. Finally, cells were labeled with 3  $\mu$ g/ml bromodeoxyuridine (BrdU) and incubated at 37 °C incubator at 5% carbon dioxide for 24 hours. The viability rates of isolated mononuclear cells were assessed using Neubauer hemocytometer and trypan blue dye method.

### *Making an animal model of stroke or brain ischemia*

14-day-old male Wistar rats were used in this study. Twenty rats were anesthetized using intraperitoneal injection of 30 mg/kg ketamine and 4 mg/kg Xylazine. Hypoxic ischemic model was created according to Hideshoshi and colleagues method. In order to, right carotid artery was closed for 30 minutes by a midline cervical incision.<sup>9, 10</sup> After exposing the common carotid, carotid artery was occluded by a string suture 6-0. Then, the skin incision was sutured under sterile conditions and animals were monitored until become conscious. On the seventh day after the hypoxia-ischemia injury, 10 neonates were anesthetized again and numbers of  $2 \times 10^5$  of stem cells were injected intravenously to each of them. The remaining 10 neonates with hypoxic conditions were considered as a sham group and were not received any stem cells. Ten healthy infants without hypoxia were considered as a control group.

### *Investigation of motor, behavioral and histological changes*

As previously mentioned, two behavioral tests were performed to evaluate motor disorders, motor coordination and somatosensory deficits in the studied animals. These tests were performed three sessions on one, seven, and 14 days after stem cell injection as follows:

In the first test, according to De Ryck and coworker's method, function of each front and hind limbs on each

side of the body was evaluated in 6 tests separately.<sup>11</sup> For each test a score of zero (no response to movement of a limb), 1 (motor incomplete or delayed more than two seconds on a limb) and 2 (a fast moving and full body) was considered that sum of scores was 16 for a healthy rat. In the second test, according to Hua and coworkers<sup>12</sup>, animals were abandoned in a corner and then count back from the left and right on 10 separate occasions and values were presented as percentage. If the mouse back from both sides equally was normal, and if mouse want to turn to one side, then it was considered indicator as a unilateral brain damage. In order to the investigation of histological changes and percentage of brain lesions, all rats were anesthetized at 14 days after the last motor test and perfused with 100 ml cold saline followed by 100 ml Paraformaldehyde 4% in PBS; their brains were removed from the skull and fixed for 24 hours in Paraformaldehyde fixative. The paraffin blocks were prepared from each brain and then were sectioned with six-micron thickness from blocks. Each 40th section of the histological series was selected and stained with hematoxylin and eosin. Then, percentage of the ischemic lesion in each section was calculated using image analysis system (Data Translation, Marlboro, MA) compared to the opposite side. For tracing transplanted stem cell in stratum, the damaged brain tissue was labeled with BrdU. The sections were reacted with anti-BrdU primary antibody and then were incubated with peroxidase-labeled secondary antibody. Finally, the sections were stained with DAB solution. The labeled cells were evaluated by a light microscope. Also to create a contrast of colors, hematoxylin was used for staining.

### *Statistical Analysis*

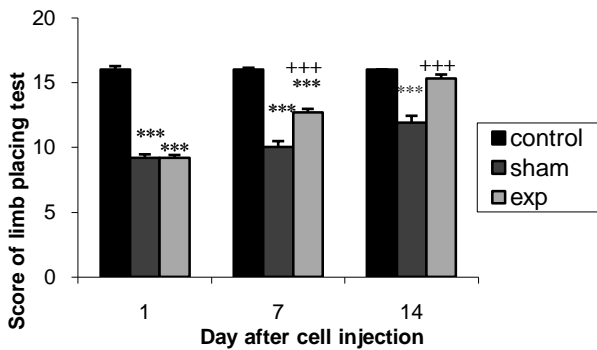
All the measured data, were analyzed using SPSS software and the means were compared with Duncan multiple range test at probability level five percent ( $P < 0.05$ ).

## Results

### *Recovery of the motor-behavior*

The results of limb placing test demonstrated that scores in the experimental animals did not differ significantly compared to the sham animals on the first day after cell injection ( $9.2 \pm 0.2$  vs.  $9.2 \pm 0.2$ , respectively). The scores were then increased on the 7 days after injection in both groups. This increase was significantly higher in the experimental group than that of the sham group ( $12.7 \pm 0.3$  vs.  $10.0 \pm 0.5$ , respectively). These scores once more increased on the 14 days after cell injection ( $15.3 \pm 0.3$  vs.  $11.9 \pm 0.5$  for the experimental and sham groups, respectively). The control group's scores of this test were 16 points in each three times. Regarding the scores obtained from the experimental and sham group, the control group had significant difference with both the experimental and the control groups in the first and seventh

days after injection. Although the difference with the sham group was continued until 14 days, no significant difference was observed between the two groups (Figure 1). In Corner Turn Test, status of parity motor in hypoxic-ischemia rats were examined by the record of the numbers turns to the right and the values were presented as percent. So, this test was performed in the one, seven, and 14 days after cell injection for all groups. There was no significant difference between scores of the experimental group ( $3 \pm \% 97$ ) compared to the sham group ( $1.3 \pm \% 98$ ) on first day after cell injection. The scores in the experimental group ( $3.7 \pm \% 75$ ) were significantly higher than that in the sham group ( $2.13 \pm \% 97$ ) on seven days after injection and continued to be significant on 14 days after cells injection ( $3.14 \pm 0.59$  vs.  $2.21 \pm 0.96$ , respectively). On the other hands, the results obtained for the control group were  $4.52 \pm \% 54$  and  $4.82 \pm \% 51$ , respectively that were different significantly on one and seven days after injection. The scores in the control group ( $4.53 \pm \% 53$ ) were significantly different compared to the experimental group on day 14 after injection (Fig. 2).



**Fig.1.**The effect of the injected stem cells derived from cord blood on Limb Placing Test score of ischemic stroke in rats. Animals in the experimental group were improved mobility in the limbs of the front and hind compared with the sham group on 14 days after injection ( $P < 0.05$ ).  
 \*\*\* $P < 0.001$  vs. control  
 +++ $P < 0.001$  vs. Sham

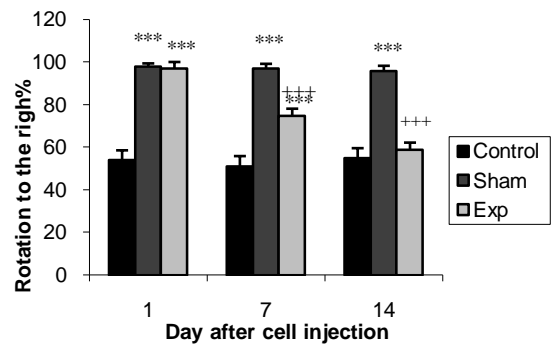
**Investigation percent of the damaged brain tissue**

Obtained results of the histological studies showed that percentage of the damaged brain tissue in the experimental group ( $21.4 \pm 3.2$ ) was significantly reduced in comparison with the sham group ( $42.2 \pm 5.7$ ; Fig. 3).

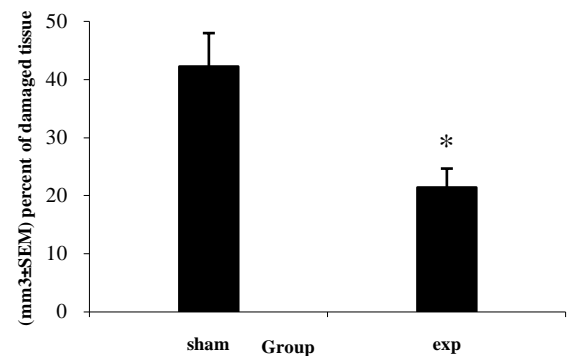
**Replacement of labeled stem cells in the damaged area**

The presence of injected stem cells in the experimental group rats was confirmed using immunohistochemistry for each group. As it is shown in Figure 4C, the migration of labeled cells with BrdU was found in the light brown area. The damaged area of the brain is separable from purple color of the background. In the untreated

hypoxic group has observed the chromatolysis phenomenon as well as cell death in the putamen and cudate nucleus (Fig. 4A-B).



**Fig. 1.**The effect of injected stem cells derived from the umbilical cord blood of rotation to the right on the Corner Turn Test in rats with ischemic stroke. Percent of turning to the right of the experimental group compared to the sham animals on 14 days after injection that shows a decreased level ( $P < 0.05$ ).  
 \*\*\* $P < 0.001$  vs. control  
 +++ $P < 0.001$  vs. sham



**Fig.3.** Percent of brain damaged tissue in comparison with healthy hemisphere in the experimental and sham groups at level less than 0.05

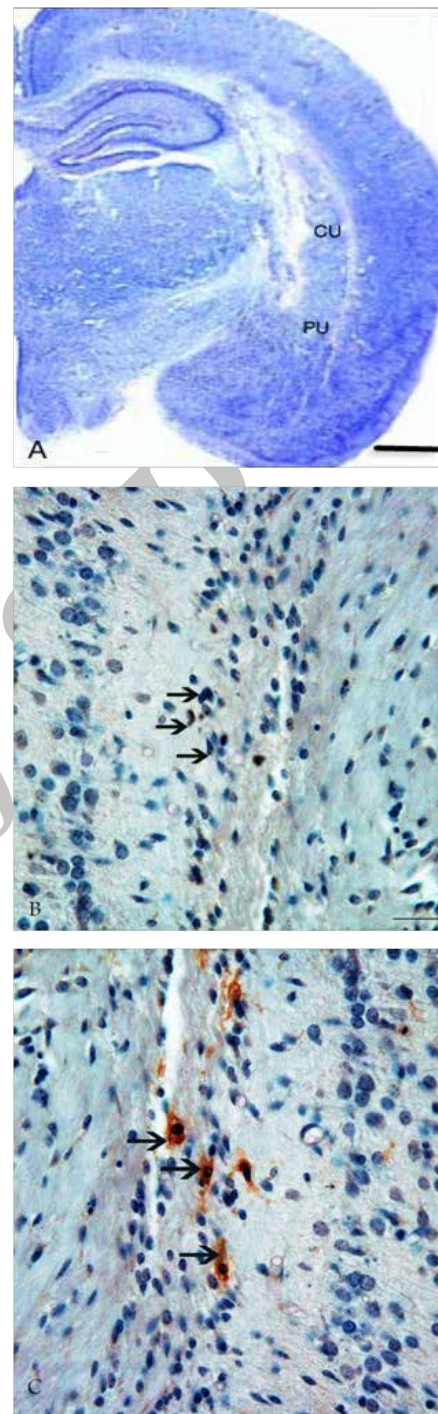
**Discussion**

Recent studies have focused on the therapeutic potential of cell transplantation in ischemic stroke. Many types of stem cells derived from human tissues have been used in experimental models of ischemic stroke, which these cells lead to recovery of motor-behavior in the animals.<sup>13</sup> Human stem cells that have investigated in these studies include: neural stem cells cultured from embryonic tissue, neural stem/progenitor cells (NPCs)<sup>14, 15</sup>, immortalization of neural cell lines<sup>16, 17</sup>, endothelial and hematopoietic precursor cells isolated from bone marrow<sup>18, 19</sup>, cord blood<sup>20, 21</sup> peripheral blood<sup>22</sup> and adipose tissue-derived stromal cells.<sup>23</sup> Several studies have reported the

benefit effects of intravenous transplantation of blood precursor cells isolated from cord blood into the brain and behavioral recovery. Regarding that human umbilical cord blood is a rich source of stem and precursor cells, Chen and colleagues were investigated survive, differentiation and improvement of motor-neurotic activity in the hypoxic- ischemic rats. In their study, rats were received intravenously  $3 \times 10^5$  cells derived from human umbilical cord blood on one and seven days after the surgery. They also done the behavioral tests (Modified Neurological Severity Score and Rotard) as well as immunohistochemistry staining for all groups. The results of the behavioral tests showed that injected cells can lead to increased scores of both tests at 24 hours after surgery, while the injected cell on 7 days caused significant changes only in points of Modified Neurological Severity Score test. In addition, immunohistochemistry staining showed the expression of neuronal and glial markers that migrate to the damaged area.<sup>24</sup>

In a similar study in 2003 by Willing and Partners, laboratory models of ischemic stroke were subjected to injection of stem cells derived from human umbilical cord blood in both intravenous and direct delivery routes at 24 hours after injury. The results of motor-behavior tests of animals showed that both types of cell transplantation were effective in improvement of the animal's spontaneous activity, but mobility recovery was observed only in the animals that were injected intravenously. They therefore concluded that administration the cells via intravenous route acts better than direct injection into the damaged tissue.<sup>25</sup> Researchers also injected different doses of the stem cells and they found that the motor-behavior recovery was dependent on the number of injected cells in the hypoxic models.<sup>26</sup>

In the present study, we investigated the effects of intravenous transplantation of the stem cells derived from umbilical cord blood of human in laboratory models of hypoxia-ischemic. Our results showed that intravenous injection of stem cells derived from umbilical cord blood causes improvement of motor-behavior in the experimental treated rats compared to the untreated ones. In addition, histological studies confirmed that the labeled cells were present at the site of injury. In this study, behavioral improvements were evaluated by two behavioral tests. For the Modified limb placing test, the control animal's points was 16 while the scores of both the experimental and sham groups had significantly difference in comparison with the control group in the first and seventh days after the cell injection. However, no difference was observed in the experimental treated animals compared to the untreated control group on 14 days after injection. For second test, the rotation of the control normal animals to the right and left was 50% on first day after treatment, while the experimental and sham groups tend to turn to the right near 100% on first day after injection.



**Fig. 4.** A) Coronal section of caudate nucleus (cu) and putamen (pu) of 21-day rat brain hemisphere in the control group with low magnification (hematoxylin & eosin staining, scale bar 40  $\mu$ ); B) Section of caudate nucleus and putamen of 21-days-old rat in the sham group (with damaged brain and without treatment) that arrows shows numbers of picnotic cells in it (scale bar=200  $\mu$ ); C) Section of caudate nucleus and putamen of 21-days-old rat in the experimental group treated with labeled stem cells that appeared as a brown color (arrow).

With look at the percents of two groups was observed significant difference compared to the control group. No difference was observed between the experimental and control groups on 14 days after injection and the percents of the rotation in the experimental animals was almost 50%. All these findings indicate that intravenous injection of the cells derived from cord blood led to improvement of motor behavior in the laboratory models. In addition, both a loss of damaged tissue in the hemisphere in the experimental group and the placement of labeled cells in the injured area confirmed that cord blood stem cells can be used for treatment of hypoxia-ischemic stroke. However, this should be considered that a hypoxic-ischemia lesion may get involved other parts of the brain like motor cortex together with the putamen and caudate nucleus. Hence, the tests do not improve motor behavior and may be relevant to other parts of the brain. It is noted that cell changes of the brain especially in putamen and caudate nucleus was evaluated in our study. Relying on the fact that cells injected intravenously is useful for the clinical treatment, it is hoped that intravenous transplantation be a guide for infants with hypoxic brain who are exposed to irreversible damages. It should be noted that more studies are required for determination of the dose of injected cells and evaluation of their benefits in a long term period for the clinical application.

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#### Ethical issues

The study was approved by the local ethics committee of Tabriz University of Medical Sciences and Ethical issues have been completely observed by the authors.

#### Conflict of interests

The authors declare no conflicts of interest.

#### References

- Zivin JA, Choi DW. Stroke therapy. *Sci Am*. 1991; 265:56–63.
- Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. *Stroke*. 2007; 38 (2):817–26.
- Schouten JW, Fulp CT, Royo NC, Saatman KE, Watson DJ, Snyder EY. A review and rationale for the use of cellular transplantation as a therapeutic strategy for traumatic brain injury. *J Neurotrauma*. 2004; 21:1501–38.
- Harris DT. Cord blood stem cells: A review of potential neurological applications. *Stem Cell Rev*. 2008; 4:269–74.
- Ormerod BK, Palmer TD, Caldwell MA. Neurodegeneration and cell replacement. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:153–70.
- Broderick JP, Brott T, Tomsick T, Huster G, Miller R. The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. *N Engl J Med*. 1992; 326:733–36.
- Kornblum HI. Introduction to neural stem cells. *Stroke*. 2007; 38 (2 Suppl):810–16.
- Kassem M, Kristiansen M, Abdallah BM. Mesenchymal stem cells: cell biology and potential use in therapy. *Basic Clin Pharmacol Toxicol*. 2004; 95:209–14.
- Sanberg PR, Willing AE, Garbuzova-Davis S, Saporta S, Liu G, Sanberg CD. Umbilical cord blood-derived stem cells and brain repair. *Ann N Y Acad Sci*. 2005; 1049:67–83.
- Hidetoshi T, Ikuko M, Hitomi OA, Kosuke A, Kazuko W. Prostaglandin D2 Protects Neonatal Mouse Brain from Hypoxic Ischemic Injury. *J Neurosci*. 2007; 27(16):4303–12.
- De Ryck M, Van Reempts J, Borgers M, Wauquier A, Janssen PA. Photochemical stroke model: flunarizine prevents sensorimotor deficits after neocortical infarcts in rats. *Stroke*. 1989; 20:1383–90.
- Hua Y, Schallert T, Keep R F, Wu J, Hoff J T, Xi G. Behavioral tests after intracerebral hemorrhage in the rat. *Stroke*. 2002; 33: 2478–84.
- Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. *Stroke*. 2007; 38(2 Suppl):817–26.
- Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, et al. Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in mongolian gerbils. *J Neurosci Res*. 2004; 78:215–23.
- Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, et al. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *PNAS*. 2004; 101:11839–44.
- Saporta S, Borlongan CV, Sanberg PR. Neural transplantation of human neuroteratocarcinoma (hNT) neurons into ischemic rats: a quantitative dose-response analysis of cell survival and behavioral recovery. *Neuroscience*. 1999; 91:519–25.
- Bliss TM, Kelly S, Shah AK, Foo WC, Kohli P, Stokes C, et al. Transplantation of hNT neurons into the ischemic cortex: cell survival and effect on sensorimotor behavior. *J Neurosci Res*. 2006; 83:1004–14.
- Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, et al. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res*. 2003; 92:692–9.
- Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol*. 2002; 174:11–20.



20. Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. **Stroke**. 2004; 35:2385–9.
21. Xiao J, Nan Z, Motooka Y, Low WC. Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury. **Stem Cells Dev**. 2005; 14: 722–33.
22. Shyu WC, Lin SZ, Chiang MF, Su CY, Li H. Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. **J Neurosci**. 2006; 26:3444–53.
23. Kang SK, Lee DH, Bae YC, Kim HK, Baik SY, Jung JS. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. *Exp Neurol*. 2003; 183:355–66.
24. Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. **Stroke**. 2001; 32(11):2682-8.
25. Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S, et al. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. **J Neurosci Res**. 2003;73(3):230-96.
26. Vendrame M, Cassady J, Newcomb J, Butler T, Penny-packer KR, Zigova T, et al. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. **Stroke**. 2004; 35(10):2390-5.

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