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Carroll J.

Georgia Health Sciences University, Augusta, GA, USA

e-mail: jcarroll@gru.edu

STEM CELLS FOR NEONATAL HYPOXIC-ISCHEMIC INJURY

ABSTRACT

Many types of adult stem cells have been used in pre-clinical situations to treat experimental hypoxic-ischemic (HI) injury in neonatal animals. Numerous laboratory reports have appeared in the literature indicating that this treatment is beneficial, and the route of cell administration does not appear to be critical. The success of treatment occurs with administration soon after the injury, and this early administration of the cells proximate to the time of injury appears to be decisive. The mechanism of benefit relates to preservation of intrinsic neurons at the site of injury rather than cell replacement by the administered cells. There are few clinical studies, and most positive reports are either from uncontrolled studies or anecdotal. Given the preclinical success with treatment, well-thought-out clinical studies need to be initiated in acutely brain injured neonates.

KEY WORDS: neonatal hypoxic-ischemic injury, stem cell transplantation, umbilical cord blood.

The potential use of stem cells for treating brain injuries in children has received widespread interest due to internet reports from families of brain injured children, news of various breakthroughs in the types of stem cells, and their success in the laboratory. We will focus on acute neonatal hypoxic-ischemic (*HI*) brain injury. This type of injury usually occurs as a result interruption of blood flow to the infant during the birth process. We will discuss the effects of stem cells in this entity because most of the experimental models attempt to mimic the damage occurring during hypoxia and ischemia in neonatal animals.

This review will deal specifically with the types of stem cells potentially available, the intrinsic regeneration which occurs in the brain normally after injury, the potential mechanisms of benefit of stem cell treatment, pre-clinical studies of stem cells with emphasis on the appropriate timing of treatment, and reports of the use of the cells in human neonates.

We previously reviewed this topic several years ago [1]. In this paper, we update the subject.

TYPES OF STEM CELLS

Stem cells are defined by their capacity for asymmetric division. Thus, they are capable of developing a dual lineage dividing into cells like themselves and other cells of a more mature type. Stem cells are generally differentiated into adult and embryonic categories, but advances in technology have served somewhat to blur this distinction.

So-called adult stem cells are those which have already undergone some degree of differentiation. These cells can be found in many tissues, but most notably bone marrow. The cells have the capacity to divide into other cells with phenotypic characteristics of cells not present in the original tissue. Generally, in respect to the bone marrow, the adult stem cells are divided into haematopoetic precursor cells and mesenchymal stem cell (*MSCs*). *MSCs* are separated from haematopoetic precursor stem cells by their adherence to plastic culture surfaces. The surface phenotype of these cells is characterized by positive staining for *CD73*, *CD90*, and *CD105*. The cells should stain negative for *CD45* and *CD34*. There have been several approaches in the manipulation of the cells, and they may even be directed to divide into neuronal cells. A well-characterized variety of these cells, which we have used in many of our experiments, are known as multipotent adult progenitor cells (*MAPCs*) [2]. Similarly, other types of *MSCs* have also been found to develop into neuronal cells.

Other interesting sources of *MSCs* in addition to bone marrow include the pulp of deciduous teeth. *Yamagata et al*. reported that adult stem cells present in teeth can protect against brain tissue loss from experimental brain injury in mice [3]. Thus, it appears that many tissues, to a greater or lesser degree, may be able to generate stem cells of some variety.

Neural stem cells are adult stem cells derived from brain; they have been proposed as having high potential for restoration in neonatal brain injury [4]. Typically, these cells are isolated from the brain of infants who have died before birth, as a result of spontaneous or elective abortion. The cells are found in the subgranular zone of the dentate gyrus and the subventricular zone. Because of their origin, in regard to elective abortion, the use of these cells is subject to ethical concerns. It is not clear how frequently these cells are utilized in clinical or experimental situations. Additionally, technical advances in the stem cell biology may serve to minimize their importance.

As an intermediary between adult stem cells and embryonic stem cells, induced pluripotent stem cells (*IPS* cells) have been derived from somatic cells such as skin fibroblasts [5]. These cells possess much the same potential as embryonic stem cells.

Embryonic stem cells have the biological advantage of being highly multipotential in terms of the types of tissue into which they can differentiate. They have the disadvantage of being subject to ethical

criticism because of their source. Once again, advances in stem cell biology may obviate the need for their usage.

Perhaps the greatest need in the field still lies at the level of basic research. We need to know more about the basic biology of stem cells. In addition to the need for knowledge in the area, the practical requirements for further clinical research are great. For example, we must know more about the long term tumorigenic potential of the different types of stem cells.

INTRINSIC REGENERATION CAPACITY OF BRAIN TISSUE

While the brain does possess some regeneration capacity after injury, this capability is severely limited. The brain's regenerative capacity has been studied in some detail with areas of neurogenesis identified with clarity. Increased neurogenesis following injury occurs in the subventricular zone with migration along the periventricular area to the hippocampus. We found new neuronal cells in the dentate gyrus in our animal injury model [6]. The new cells that appear first are either microglial or endothelial. The areas of greatest numbers of new neurons are the subventricular zone, the dentate gyrus and the *CA1* area of the hippocampus.

Further, the relation between this cellular phenomenon and the clinical improvement that occurs after injury is not known. Undoubtedly, the new cells make a favorable contribution, but it is insufficient for recovery. Our clinical experience confirms that brain injuries of any significance are permanent.

The fact that there is some endogenous capacity for regeneration does indeed hold out the promise for enhancing this process. This may be possible with more knowledge about the stem cell secretory factors that allow survival of greater numbers of neurons [7].

PRE-CLINICAL EXPERIMENTAL DATA

The following Table shows a number of pre-clinical experiments demonstrating the success with various type of stem cell transplantation in acutely injured neonatal animals. All but one of the studies utilized the standard HI model with ligation of one carotid artery followed by a period of hypoxia [8]. The table does not show all the experiments reported in the literature, as there are many, but the trends are shared among all reports.

All of the above reports deal with acute injury with brief intervals between the injury and the treatment. The cell types used are variants of MSCs. Interestingly, the route of administration does not seem to matter. Even nasal administration may be a viable route [9, 18].

In unpublished work we used human-derived *IPS* cells, which had been allowed to transform to neuroprogenitor cells. These cells were injected into the hippocampus eight days after neonatal *HI* injury. Behavioral improvement and increased neuronal cell survival were noted.

The conclusion from these pre-clinical, animal experiments is that adult stem cell treatment is beneficial when administered shortly after acute brain injury. Further, the cells may be administered by a variety of routes, all leading to a beneficial result.

MECHANISM OF ACTION

We have recently reviewed this topic [19]. The main idea concerning the putative benefit of stem treatment is that the cells would replace dead or damaged cells. The secondary hypothesis is the possible protective effect of the stem cells or whatever they secrete as a way of preserving intrinsic cells [20]. Cell replacement would seem to be the ideal mechanism in this situation. However, only small numbers of transplanted cells survive, and most of these cells do not transform into neurons [15, 21]. The transplanted cells that do survive generally do not develop neuronal processes which are sufficient for normal activity [22].

The more likely benefit has to do with the greater survival of intrinsic neuronal cells. Certain growth factors, such as nerve growth factor and brain-derived neurotrophic factor, are increased by the injection of *MSCs*. *Yamagata et al*. demonstrated that stem cells from exfoliated teeth inhibited the expression of proinflammatory cytokines, increased the expression of anti-inflammatory cytokines and reduced apoptosis, all leading to increased intrinsic cell survival [3]. The process may be mediated by regulation of neuronal cell gene expression [23]. In another experiment dealing with neural stem cells, microarray analysis revealed upregulation of genes involved in neurogenesis and neurotrophic elements [24].

Several other mechanisms probably play a role. Human umbilical cord cells may reduce cell death in neonatal brain injury by attenuating reactive gliosis [25]. We have previously reviewed the process of blood vessel regeneration in acute brain injury [19]. Stem cells may have an effect on the spleen which benefits acute brain injury. Several groups have shown that injection of adult stem cells diminishes the release of inflammatory cells from the spleen [26, 27]. It is thought that these splenic inflammatory cells play a deleterious role in the destructive process occurring in injured brain.

Finally, in contrast to most other reports, it has been reported that *MSCs* have been shown to assist in cortical rewiring after neonatal brain injury [28].

Table 1. Pre-Clinical Experiments in Neonatal Rodents.

MODEL	TIME AFTER INJURY	CELL TYPE	ROUTE	REF.
HI	3, 7, or 10 days	MSC	Intranasal	[9]
HI	3,7, or 14 days	NSC	Intra-arterial	[10]
HI		MSC-NP	Brain	[11]
HI	3 days	MSC	Intra-cardiac	[12]
HI	1 day	Umbilical cord	Intravenously	[13]
Middle cerebral a. occlusion	1 day	Umbilical cord blood MSC	Intraventricular	[14]
HI	1 day	Umbilical cord blood MSC	Intraperitoneal	[15]
HI	7 days	MAPC	Intrahippocampus	[16]
HI	7 days	MAPC	Intravenously	[17]

CLINICAL DATA

Very few reports are currently in the literature dealing with the use of stem cells in acute brain injury. Six children were reported from China. These children received human neural precursor cells within 4 to 20 days after the injury. One child had carbon monoxide poisoning, one had severe hypoglycemia, and the four others had severe neonatal asphyxia. The treatment cells were derived from a 12-week fetus after spontaneous abortion. The cells were injected into the lateral ventricles. The authors reported that all patients improved the second day after transplantation, and four of the patients reached the normal level of development. No complications were reported [29].

In another Chinese report of a single infant who had experienced severe *HI* encephalopathy, the same type of human neural precursor cells were injected into the lateral ventricle. The infant was said to reach the normal level of development 28 days after the transplant [30].

We are aware of only one current study, at *Duke University* in the USA, which is designed to determine the possible benefit of acute stem cell treatment of acute brain damage in the neonate (clinical trial *ID NCT00593242*). The source of the stem cells is autologous umbilical cord blood. No results have been reported.

The lack of clinical data from blinded, controlled studies frustrates our knowledge in the field.

FUTURE POSSIBILITIES

Two lines of research need to come together in order to further the apparent potential of stem cell therapy for acute neonatal brain injury.

First, more work must be done in the area of stem cell biology, both at the basic and pre-clinical level. While we know a considerable amount about the different types of stem cells, we do not yet know which varieties have the greatest potential in the therapeutic arena. In order to achieve this component, preclinical testing of stem cells should be accomplished by comparing the efficacy and safety of the types in animal models. Careful analyses of the behavioral and tissue outcomes should be examined.

Second, blinded, controlled studies need to be conducted in groups of human infants who have comparable clinical features. These studies are difficult and expensive to accomplish and require follow-up of the children over extended periods. Such a systematic approach is often frustrating to both parents and researchers. But these studies are needed in order to achieve an evidenced-based approach to treatment.

CONCLUSIONS

STEM CELL TRANSPLANTATION HOLDS GREAT PROMISE IN TREATING ACUTE BRAIN INJURIES AND MOST SPECIFICALLY *HI* INJURIES IN NEONATES. MOST OF THE PRE-CLINICAL MODELS REPORTING SUCCESS HAVE STUDIED THIS ISSUE. THE MECHANISM LEADING TO BENEFIT APPEARS TO BE NEUROTROPHIC AND NEUROPROTECTIVE RATHER THAN NEURON REPLACEMENT. MORE BASIC RESEARCH IN STEM CELL BIOLOGY IS NEEDED.

WELL-DESIGNED CLINICAL STUDIES NEED TO BE CONDUCTED. AN OBVIOUS CHOICE FOR THE CELL TYPE TO BE USED, BECAUSE OF ITS HIGH DEGREE OF SAFETY, IS AUTOLOGOUS UMBILICAL CORD BLOOD. GIVEN THE REQUIREMENT THAT THESE STUDIES MUST BE CONDUCTED VERY SOON AFTER THE INJURY, THE ORGANIZATION AND CONDUCT OF THE STUDIES WILL BE EXPENSIVE AND ARDUOUS.

REFERENCES

1. *Carroll J., Borlongan C.* Adult Stem Cell Therapy for Acute Brain Injury in Children // CNS & Neurological Disorders – Drug Targets. – 2008. – **7**. – P. 1-8.
2. *Keene C., Ortiz-Gonzalez X., Jiang Y. et al.* Neural differentiation and incorporation of bone marrow-derived multipotent adult progenitor cells after single cell transplantation into blastocyst mouse embryos // Cell Transplantation. – 2003. – **12**. – P. 201-213.
3. *Yamagata M., Yamamoto A., Kako E. et al.* Human dental pulp-derived stem cells protect against hypoxic-ischemic injury in neonatal mice // Stroke. – 2013. – **44**. – P. 551-554.
4. *Lee I., Jung K., Kim M. et al.* Neural stem cells: properties and therapeutic potentials for hypoxic-ischemic brain injury in newborn infants // Pediatrics International. – 2010. – **52**. – P. 855-65.
5. *Takahashi K., Yamanaka S.* Induced pluripotent stem cells in medicine and biology // Development. – 2013. – **140**. – P. 2257-2267.
6. *Bartley J., Soltau T., Wimbourne H. et al.* BrdU-positive cells in the neonatal mouse hippocampus following hypoxic-ischemic brain injury // BMC Neuroscience. – 2005. – **6**. – P. 63-74.
7. *Donega V, van Velthoven CT, Nijboer CH, et al.* The endogenous regenerative capacity of the damaged newborn brain: neurogenesis with mesenchymal stem cell treatment // J. Cerebral Blood Flow & Metabolism. – 2013. – **33**. – P. 625-634.
8. *Rice J., Vannucci R., Brierly J.* The influence of immaturity on hypoxic-ischemic brain damage in the rat // Ann Neurol. – 1981. – 9. – P. 131-141.
9. *Donega V., van Velthoven C., Nijboer C. et al.* Intranasal mesenchymal stem cell treatment for neonatal brain damage: long-term cognitive and sensorimotor improvement // PLoS ONE. – 2013. – **8**. – P. e51253.
10. *Rosenblum S., Wang N., Smith T. et al.* Timing of intra-arterial neural stem cell transplantation after hypoxia-ischemia influences cell engraftment, survival, and differentiation // Stroke. – 2012. – **43**. – P. 1624-1631.
11. *Park S, Koh SE, Maeng S, et al.* Neural progenitors generated from mesenchymal stem cells of first-trimester human placenta matured in the hypoxic-ischemic rat brain and mediated restoration of locomotor activity // Placenta. – 2011. – **32**. – P. 269-276.
12. *Lee J., Kim B., Jo C., et al.* Mesenchymal stem-cell transplantation for hypoxic-ischemic brain injury in neonatal rat model // Pediatric research. – 2010. – **67**. – P. 42-46.
13. *de Paula S., Greggio S., Marinowic D. et al.* The dose-response effect of acute intravenous umbilical cord blood cells on brain damage and neonatal hypoxia-ischemia // Neuroscience. – 2012. – **210**. – P. 43-66.
14. *Kim E., Ahn S., Im G. et al.* Human umbilical cord blood-derived mesenchymal stem cell transplantation attenuates severe brain injury by permanent middle cerebral artery occlusion in newborn rats // Pediatric Research. – 2012. – **72**. – P. 277-84.
15. *Meier C., Middelanis J., Wasielewski B. et al.* Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells // Pediatric Research. – 2006. – **59**. – P. 244-249.

16. *Yasuhara T., Matsukawa N., Yu G. et al.* Transplantation of cryopreserved human bone marrow-derived multipotent adult progenitor cells of neonatal hypoxic-ischemic injury: targeting the hippocampus // Reviews in the Neurosciences. – 2006. – **17**. – P. 215-225.
17. *Yasuhara T., Hara K., Maki M. et al.* Intravenous grafts recapitulate the neurorestoration afforded by intracerebrally delivered multipotent adult progenitor cells in neonatal hypoxic-ischemic rats // J. Cerebral Blood Flow and Metabolism. – 2008. – **28**. – P. 1804-1810.
18. *van Velthoven C., Kavelaars A., van Bel F., Heijnen C.* Nasal administration of stem cells: a promising novel route for ischemic brain damage // Pediatric Research. – 2010. – **68**. – P. 419-422.
19. *Carroll J.* Human cord blood for the hypoxic-ischemic neonate // Pediatric Research. – 2012. – **71**. – P. 459-63.
20. *Van Velthoven C., Kavelaars A., Heijnen C.* Mesenchymal stem cells as a treatment for neonatal ischemia // Pediatric Research. – 2012. – **71**. – P. 474-481.
21. *Erices A., Conget P., Minguell J.* Mesenchymal progenitor cell in human umbilical cord blood // British Journal of Haematology. – 2000. – **109**. – P. 235-242.
22. *Zhao L., Duan W., Reyes M. et al.* Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats // Experimental Neurology. – 2002. – **174**. – P. 11-20.
23. *van Velthoven C., Kavalaars A., van Bel F. et al.* Mesenchymal stem cell transplantation changes the gene expression of the neonatal ischemic brain // Brain, Behavior & Immunity. – 2011. – **25**. – P. 1342-1348.
24. *Daadi M., Davis A., Arac A. et al.* Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. – Stroke. – 2010. – **41**. – P. 516-523.
25. *Wasielewski B., Jensen A., Roth-Harer A. et al.* Neuroglial activation and CX43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury // Brain Research. – 2012. – **1487**. – P. 39-53.
26. *Borlongan C., Lind J., Dillon-Carter O. et al.* Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats // Brain Research. – 2004. – **1010**. – P. 108-116.
27. *Robinson S., Niu T., de Lima M. et al.* Ex vivo expansion of umbilical cord blood // Cytotherapy. – 2005. – **7**. – P. 243-250.
28. *van Velthoven C., van de Lootj Y., Kavalaars A. et al.* Mesenchymal stem cells restore cortical rewiring after neonatal // Annals of Neurology. – 2012. – **71**. – P. 785-796.
29. *Luan Z., Liu W., Qu S. et al.* Treatment of newborns with severe injured brain with transplantation of human neural precursor cells // Zhonghua Erke Zazhi. – 2011. – **49**. – P. 445-449.
30. *Luan Z., Yin G., Hu X. et al.* Treatment of an infant with severe neonatal hypoxic-ischemic encephalopathy sequelae with transplantation of human neural stem cells into cerebral ventricle // Zhonghua Erke Zazhi. – 2005. – **43**. – P. 580-583.