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Cord Blood Stem Cells: A Review of Potential Neurological Applications

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Abstract It is estimated that as many as 128M individuals in the United States, or 1 in 3 people, might benefit from regenerative medicine therapy. Many of these usages include applications that affect the nervous system, including cerebral palsy, stroke, spinal cord injury and neurodegenerative disease such as Parkinson's. The numbers of such individuals affected range from 10,000 (for cerebral palsy) to 700,000 annually (for stroke) at a cost of more than $65B. For the foreseeable future, regenerative medicine entrée to the clinic will depend upon the development of adult or non-embryonic stem (ES) cell therapies. Currently, non-ES cells easily available in large numbers from affected individuals can be found in the bone marrow, adipose tissue and umbilical cord blood (CB). It is our belief that CB stem cells are the best alternative to ES cells as these stem cells can be used to derive tissues from the mesodermal, endodermal and ectodermal germ lineages. CB contains a mixture of different types of stem cells in numbers not seen in any other location including embryonic-like stem cells, hematopoietic stem cells, endothelial stem cells, epithelial stem cells, mesenchymal stem cells and unrestricted somatic stem cells. This review will summarize the findings reported in the literature with regards to the use of CB stem cells to neurological applications including in vitro work, pre-clinical animal studies, and patient clinical trials.

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Background

Since its initial clinical use in 1989, umbilical cord blood (CB) (or merely, cord blood) has been considered an interchangeable alternative to bone marrow and mobilized peripheral blood, and merely another source of blood (hematopoietic) stem cells. As of 2007, more than 8,000 CB transplants have been performed worldwide [1], and numerous public and private agencies have emerged to store CB for public or familial use, respectively. In addition to being readily available, CB has other distinct advantages over bone marrow and peripheral blood stem cells for hematopoietic transplantation, including greater tolerance for HLA-mismatches between donor and recipient and decreased graft-versus-host disease [2, 3]. Recently, it has shown that CB stem cells have the ability to regenerate numerous tissue types, and when transplanted into animals and humans, have produced measurable functional improvements [4, 5]. Generally, tissue-derived stem cells have been described for neural [6], muscle [7], retinal [8], pancreas [9], skin [10] and liver tissues [11] but these tissue specific stem cells have limited self-renewing capabilities and are unable to reconstitute a whole organ system. However, CB stem cells appear to be unique in its ability to undergo pluripotential differentiation. A previous study reported the identification and isolation of pluripotent, unrestricted somatic stem cells (USSC) from human CB. When cultured in vitro, USSC differentiated into osteoblasts, chondroblasts, adipocytes, and neural cells [12], which putatively identifies USSCs as a novel population of

mesenchymal stem cells (MSC). Dr. Colin McGuckin and colleagues demonstrated that primitive, embryonic-like stem cells could also be isolated from human CB. This work identified very primitive stem cells that were able to differentiate into hepatic progenitor and pancreatic islet cells. [13].

From birth until old age, we are at risk of neurological damage from a variety of known and unknown assaults. It is estimated in the United States that approximately 10,000 infants are born annually with cerebral palsy (or 1 in 400 births), accruing lifetime medical costs of $11.5B for all affected individuals [4]. An additional 130,000 individuals suffer spinal cord injuries annually in the United States (both young and old) with approximate $2M annual lifetime medical costs [5]. As the population moves into middle age (40 years and upwards) the risk of stroke (both embolic and hemorrhagic) increases to the point of 700,000 cases annually (and 250,000 deaths) with a medical burden of over $64B [14]. Finally, in late middle- and old-age individuals are faced with the prospect of neurodegenerative diseases such as Parkinson's [60,000 new cases annually, annual cost of $24B; 4].

Most of the therapies for these diseases are palliative rather than restorative, greatly impacting the quality of life for affected individuals, as well as the medical burden on society. The use of stem cells in regenerative medicine however, holds the promise of replacing or regenerating these affected neurological tissues. However, success will depend upon selection of the correct stem cell source and proper utilization. This review will attempt to demonstrate that CB stem cells are not only able to generate nervous tissue in the laboratory, but also that use of such CB stem cells in pre-clinical animal models has successfully treated a variety of such diseases, and has led to the successful implementation of human clinical trials.

CB and Pluripotentcy

Over the past several years there have been many reports of the isolation and characterization of multipotential cells from CB. Early studies implying the pluipotential differentation capacity of CB stem cells was demonstrated by work from the laboratories of Gaballa [15] and Nichols [16–18], which showed that CB stem cells could be used in vivo to derive endothelial and epithelial tissues, respectively. Gaballa and colleagues showed that intramyocardial and intravenous delivery of CB improved vascularization and cardiac function after experimental myocardial infarction (MI) in rats. Human nuclei were detected mainly in the endothelium of the treated animals, resulting in significant angiogenesis. CB stem cells led to an increase in blood vessel density and improved left ventricular remodeling after ischemia and reperfusion. Work from the group of Nichols et al. [16–18] used purified CD34+ and CD34− CB stem cells as a viable therapeutic modality for the treatment of ocular surface disease. Histology and immunohistochemistry of the differentiated CB stem cells revealed that the resultant cell sheet was morphologically indistinguishable from corneal epithelial cells. CB stem cells were capable of expressing the corneal epithelial specific cytokeratin, K3. Significantly, upon transplantation into large animals, the CB-derived tissues behaved as physiological corneas.

In terms of specific neurological applications McGuckin et al [19] demonstrated the presence of embryonic stem (ES)-like stem cells in CB that could be expanded for up to 8 weeks in culture. These cells could be differentiated to exhibit neuronal cell morphology as well as express neuronal markers (e.g., GFAP, nestin, musashi-1, and nectin). These neuronal-like cells also released glial-derived neurotrophic factor into the cultures. Jang et al [20] showed that purified CD133+ CB stem cells upon exposure to retinoic acid differentiated into neuronal (astrocytes and oligodendrocytes) and glial cells that expressed neuronal markers (including tubulin βIII, neuron specific enolase, NeuN, microtubule-associated protein-2 (MAP2) and the astrocyte-specific marker glial fibrillary acidic protein). Further, non-hematopoietic stem cells found in CB (most likely MSC) also could become neural-like cells in culture, i.e., astrocytes and oligodendrocytes [21]. In confirmation of these reports, work from Harris and Ahmad [22] has also shown that CD133+ and Lin- populations isolated from CB could become glial cells, astrocytes and oligodendrocytes in vitro.

Rogers and colleagues have isolated a population of CD45+ cells from CB that can develop developmental properties similar to the CD45-negative MAPC, USSC and MPC that have been described by others [23]. These multipotential stem cells (MPSC) were isolated by culture in a serum free, growth factor supplemented medium (SCF + FL + FGF). MPSC were observed to express the stem cell markers Oct-4 and Nanog, the early tissue developmental markers nestin, desmin, GFAP and Cfab1; and were capable of differentiating into bone, muscle, neural, blood and endothelial cells after exposure to specialized differentiation media [24, 25]. MPSC also expressed other stem cell markers such as SSEA3 and SSEA4 [26]. Multi-potential CD45+ blood cells have also been reported by others. Zhao et al has also reported the isolation of an adherent, CD45+ multi-potential cell from CB [27]. Work from the Rogers' laboratory demonstrated that neural cell differentiation of these MPSC resulted in the expression of nestin and neurofilament very early during culture in a high percentage of cells. The full range of neural differentiation ability of the MPSC was demonstrated by the achievement of positive phenotypic and functional indicators for dopaminergic neurons, oligodendrocytes and astrocytes [23].

The utility of CB stem cell populations for use in cell-based treatments of brain injuries and neurological diseases

has recently been reviewed by Chen et al [28, 29]. We will highlight some of the more significant findings below.

CB and Stroke

Cerebrovascular diseases remain the third leading cause of death in the United States, not including the multitudes of individuals who survive only to suffer debilitating lifelong injuries. Cerebral ischemia is by far the most prevalent cause of stroke (87%, AHA). Approximately 700,000 people in the United States affected by stroke annually; 1 in 16 Americans who suffer a stroke will die from it [14]. The brain is extremely sensitive to hypoxia and some degree of tissue death is likely from stroke. At a relatively young age the brain loses most of its plasticity so any significant tissue death can be profoundly devastating. Interestingly, in young children the brain is very plastic and very large portions of the brain can be removed (such as removal of tumors or hemispherectomy for severe seizures) with relatively low to no noticeable long term neurological damage. These facts suggest that younger more naïve cells, which could be generated by differentiated from CB, might have a greater capacity to regenerate the injured brain.

Nowhere has the potential significance of CB stem cell therapy for the treatment of neurological disease been greater than in the area of stroke therapy. As early as 2001, it was demonstrated that the infusion of CB stem cells into rats with the commonly used MCAO (medial carotid artery occlusion) model of stroke could ameliorate many of the physical and behavioral deficits associated with this disease [29]. Studies demonstrated that direct injection of the stem cells into the brain was not required [30], and in fact, beneficial effects could be observed even if the stem cells did not actually make their way into the target organ (probably via the release of growth and repair factors triggered by anoxia) [31, 32]. The beneficial effects seemed to be dose-dependent and could reduce the size of the infarcted tissue [33]. Once again, it appeared that multiple progenitor populations may be capable of mediating these effects [34]. Significantly, unlike current pharmacological interventions that require treatment in the first few hours after stroke, CB stem cell therapies were still effective up to 48 h after the thrombotic event [35]. In fact, administration of CB stem cells immediately after the ischemic event may be detrimental in that the inflammatory milieu may be toxic to the administered stem cells.

In fact, the majority of reported studies [34–41] have shown that CB administration in stroke resulted in some degree of therapeutic benefit with no adverse effects. Neuroprotective effects [34–36, 39, 40, 42] as well as functional/behavioral improvements [34, 35, 40, 41] of CB therapies have been widely reported. Neurological improvement was accompanied decreased inflammatory cytokines [39], neuron rescue/reduced ischemic volume [34–36], as well as lowered parenchymal levels of granulocyte and monocyte infiltration and astrocytic and microglial activation [35]. Thus, the mechanisms behind the observed beneficial effects afforded by CB therapies included reduced inflammation [36], apoptotic protection [34] and a combination of trophic actions and nerve fiber reorganization [34]. This later postulation is particularly encouraging as it implies that CB therapy can mediate both direct restorative effects to the brain as well as tropic neuroprotection. Many of the studies lend support to this tropic role, in that several investigators reported [34, 39, 41] neural protection with little to no detection of CB cells engrafted in the brain. The level of engraftment in the brain appeared to be a function of the route of CB administration. When CB was administered intravenously [39, 41–43], little or no CB migration to the brain was found. However, when CB was given intraperitoneally [43] there was evidence of neural restorative effects. Two studies investigated the optimal delivery time of cells.

Early studies have also shown benefit in animal models of hemorrhagic (as opposed to embolic) stroke [40]. For additional information one is referred to the recent review on cell therapies for stroke found in reference [44].

Other Neurological Applications

Neurological repair has a much more complex etiology than many other conditions being evaluated for stem cell-based therapy. However, in animal models of stroke, amyotrophic lateral sclerosis, Parkinson's disease, cerebral palsy and spinal cord injury CB stem cell infusion has resulted in observable behavioral improvement compared to control animals [19, 21–23, 29–35, 37, 40, 44–49, 50–51]. The same beneficial effect has also been observed for animals with traumatic brain injury.

As CB stem cells have the ability to become different types of nervous cells, it may be possible to extend its use to other areas of neurological damage, including spinal cord injury. In fact, spinal cord injured rats infused with CB stem cells showed significant improvements 5 days post-treatment compared to untreated animals. The CB stem cells were observed at the site of injured nervous tissue but not at uninjured regions of the spinal cord [53]. This finding is supported by studies showing that CB stem cells transplanted into spinal cord injured animals differentiated into various neural cells, thereby improving axonal regeneration and motor function [47]. Similar results have been reported by Rogers et al, which demonstrated using a compression model of spinal cord injury the presence of CB stem cell early but late in the therapy implying a trophic effect of such interventions (personal communication). Significantly, in a recently reported clinical use of CB stem cells to treat a patient with a spinal cord injury [48] it was stated that transplantation of CB cells improved her sensory perception

and mobility in the hip and thigh regions. Both CT and MRI studies revealed regeneration of the spinal cord at the injury site. To date however, this study has not been replicated by other laboratories.

Lu et al [49] have demonstrated that intravenous administration of CB could be used to treat traumatic brain injury in a rat model. In this model the CB cells were observed to enter the brain, selectively migrate to the damaged region of the brain, expressed neural markers once *in situ*, and reduced the neurological damage. Similarly, CB stem cell transplantation alleviated symptoms of newborn cerebral palsy in a rat model, with improved neurological effects [37]. An additional study found that intraperitoneal administration of CB, in a somewhat clinically relevant setting (24 h post-CP diagnosis), produced an alleviation of the neurological affects of CP, including a reduction of spastic paresis and an increase in normal walking behavior.

Other investigators have shown that CB stem cells are effective therapies for Parkinson's disease [50, 51, 54, 55]. In both animal models CB stem cell infusion delayed symptom onset and progression, as well as prolonged survival.

Finally, two recent pre-clinical animal studies are of significant interest. In the first study, Bachstetter et al [56] demonstrated that intravenous injections of CB mononuclear cells could stimulate neurogenesis in the brains of aged rats, as evidenced by a variety of histological analyses. The mechanism of action was postulated to be a rejuvenation of the aged brain microenvironment mediated via a decrease in inflammation (i.e., cytokines) and a decrease in activated microglia. It will be of interest to learn if such a therapy is correlated with improvements in cognitive function. In the second study, Nikolic et al [57] demonstrated that intravenous administration of CB stem cells could modify the progression of an Alzheimer's disease animal model. That is, a marked reduction in A*beta* levels/*beta*-amyloid plaques and associated astrocytosis was observed following multiple low-dose infusions of CB stem cells in the Tg2576 AD mouse model.

Clinical Neurological Applications of CB Stem Cells

Information gained from the laboratory and pre-clinical animal studies are now being translated into clinical applications, albeit somewhat limited in scope. CB stem cell infusion has started to make its way into the clinic to treat patients with neurological damage. CB Registry (a family CB stem cell bank) has recently released 15 CB stem cell samples for autologous use in the treatment of cerebral palsy at a clinical trial being conducted at Duke University (see http://www.cordblood.com). Preliminary results have been significant and encouraging (see http://www.msnbc.msn.com/id/23572206/), and many additional patients (up to 40) are being enrolled. Further, similar results have been reported recently by investigators treating children in Europe and Asia (personal communication, Novussanguis Foundation, Paris, France, May 2008). The University of Texas at Houston is to begin a FDA-approved clinical trial to treat children with traumatic brain injury utilizing autologous CB stem cell infusions (Cox & Baumgartner, 2008, UT Health Sciences Center, Houston, Texas), based on the successful results obtained with a similar autologous bone marrow stem cell study. Further, a 300 patient trial for spinal cord injury was begun in China in 2007 (according to a recent AABB SmartBrief update), and it has been recently suggested that CB stem cells will be used for the clinical treatment of stroke.

Conclusions

Regenerative medicine offers the hope of remedial therapy, if not a cure, for many degenerative diseases, including those of neurological origin. However, in order to make this possibility a reality, one must have available a source of stem cells derived from the patient, the stem cells must be available in large numbers, and the process must be economical. Thus, for the foreseeable future patient access to regenerative medicine will depend upon the development of adult or non-ES cell therapies. It is our belief that CB stem cells are the best alternative to ES cells as these stem cells can be used to derive tissues from all three (mesodermal, endodermal and ectodermal) germ lineages. CB contains a mixture of different types of stem cells in numbers not seen in any other location including embryonic-like stem cells, hematopoietic stem cells, endothelial stem cells, epithelial stem cells, and MSC. Extensive published work from multiple investigators has demonstrated that CB stem cells are amenable to neurological applications including as evidenced by in vitro studies, pre-clinical animal models of disease, and more recently by patient clinical trials. Therefore, umbilical CB stem cells are unique in their ability to be used for stem cell transplantation in the treatment of blood disorders, as well as use in regenerative medicine to treat patients with neurological disease.

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References

1. Rubinstein, P. (2006). Why cord blood? *Human Immunology*, *67*, 398–404.
2. Rubinstein, P., Rosenfield, R. E., Adamson, J. W., et al. (1993). Stored placental blood for unrelated bone marrow reconstitution. *Blood*, *81*, 1679–1690.

3. Gluckman, E., Rocha, V., & Boyer-Chammard, A. (1997). Outcome of cord-blood transplantation from related and unrelated donors. *New England Journal of Medicine*, *337*, 373–381.
4. Harris, D. T., Badowski, M., Ahmad, N., & Gaballa, M. (2008). The potential of cord blood stem cells for use in regenerative medicine. *Expert Opinion on Biological Therapy*, *7*(9), 1311–1322.
5. Harris, D. T., & Rogers, I. (2007). Umbilical cord blood: a unique source of pluripotent stem cells for regenerative medicine. *Current Stem Cell Research & Therapy*, *2*, 301–309.
6. Seaberg, R. M., & van der Kooy, D. (2002). Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. *Journal of Neuroscience*, *22*, 1784–1793.
7. Hill, E., Boontheekul, T., & Mooney, D. J. (2006). Regulating activation of transplanted cells controls tissue regeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 2494–2449.
8. Tropepe, V., Coles, B. L., Chiasson, B. J., Horsford, D. J., Elia, A. J., McInnes, R. R., et al. (2000). Retinal stem cells in the adult mammalian eye. *Science*, *287*, 2032–2036.
9. Seaberg, R. M., Smukler, S. R., Kieffer, T. J., Enikolopov, G., Asghar, Z., Wheeler, M. B., et al. (2004). Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nature Biotechnology*, *22*, 1115–1124.
10. Toma, J. G., Akhavan, M., Fernandes, K. J., Barnabe-Heider, F., Sadikot, A., Kaplan, D. R., et al. (2001). Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nature Cell Biology*, *3*, 778–784.
11. Yoon, B. I., Choi, Y. K., & Kim, D. Y. (2004). Differentiation processes of oval cells into hepatocytes: proposals based on morphological and phenotypical traits in carcinogen-treated hamster liver. *Journal of Comparative Pathology*, *131*, 1–9.
12. Kogler, G., Sensken, S., & Wernet, P. (2006). Comparative generation and characterization of pluripotent unrestricted somatic stem cells with mesenchymal stem cells from human cord blood. *Experimental Hematology*, *34*(11), 1589–95.
13. McGuckin, C., Forraz, N., Baradez, M. O., et al. (2005). Production of stem cells with embryonic characteristics from human umbilical cord blood. *Cell Proliferation*, *38*, 245–255.
14. Copeland, N., Harris, D., & Gaballa, M. A. (2008). Human umbilical cord blood stem cells are a beneficial therapy in experimental models of myocardial infarction and stroke. *Clinical Medicine: Cardiology*, in press.
15. Sunkomat, J. N. E, Goldman, S., Harris, D. T., et al. (2008). Cord blood-derived MNCs delivered intracoronary contribute differently to vascularization compared to CD34+ cells in the rat model of acute ischemia. Manuscript submitted for publication.
16. Harris, D. T., He, X., Camacho, D., Gonzalez, V., & Nichols, J. C. (2006). The potential of cord blood stem cells for use in tissue engineering of the eye, stem cells & regenerative medicine, Jan 23–25, 2006, San Francisco, Abstract
17. Harris, D. T., He, X., Badowski, M., & Nicols, J. C. (2008). Regenerative medicine of the eye: a short review. In N. Levicar, N. A. Habib, I. Dimarakis, & M. Y. Gordon (Eds.), *Stem cell repair & regeneration*(vol. 3). London: Imperial College Press.
18. Nichols, J. C., He, X., & Harris, D. T. (2005). Differentiation of Cord Blood Stem Cells Into Corneal Epithelium. *Invest Ophthalmol Vis Sci*, *46*, E-Abstract 4772.
19. Mcguckin, C. P., Forraz, N., Allouard, Q., & Pettengell, R. (2004). Umbilical cord blood stem cells can expand hematopoietic and neuroglial progenitors in vitro. *Experimental Cell Research*, *295*, 350–359.
20. Jang, Y. K., Park, J. J., Lee, M. C., et al. (2004). Retinoic acid-mediated induction of neurons and glial cells from human umbilical cord-derived hematopoietic stem cells. *Journal of Neuroscience Research*, *75*, 573–584.
21. Buzanska, L., Jurga, M., Stachowiak, E. K., Stachowiak, M. K., & Domanska-Janik, K. (2006). Neural stem-like cell line derived from a nonhematopoietic population of human umbilical cord blood. *Stem Cells Develop*, *15*, 391–406.
22. Harris, D. T., Ahmad, N., Saxena, S. K. et al. (2005). The Potential of Cord Blood Stem Cells for Use in Tissue Engineering. Abstract, Intl. TESi meeting, Shanghai, China, Oct 2005
23. Rogers, I., Yamanaka, N., Bielecki, R., Wong, C. J., Chua, S., Yuen, S., et al. (2007). Identification and analysis of in vitro cultured CD45-positive cells capable of multi-lineage differentiation. *Experimental Cell Research*, *313*, 1839–1852.
24. Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., et al. (2003). The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell*, *113*, 631–642.
25. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, *126*, 663–676.
26. Tippett, P., Andrews, P. W., Knowles, B. B., Solter, D., & Goodfellow, P. N. (1986). Red cell antigens P (globoside) and Luke: identification by monoclonal antibodies defining the murine stage-specific embryonic antigens -3 and -4 (SSEA-3 and SSEA-4). *Vox Sang*, *51*, 53–56.
27. Yu, M., Xiao, Z., Shen, L., & Li, L. (2004). Mid-trimester fetal blood-derived adherent cells share characteristics similar to mesenchymal stem cells but full-term umbilical cord blood does not. *British Journal of Haematology*, *124*, 666–675.
28. Schmidt, D., Breymann, Y., Weber, A., et al. (2004). Umbilical cord blood derived endothelial progenitor cells for tissue engineering of vascular grafts. *Soc Thorac Surg*, *78*, 2094–2098.
29. Chen, J., Sanberg, P. R., Li, Y., et al. (2001). Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke*, *32*, 2682–2688.
30. Willing, A. E., Lixian, J., Milliken, M., et al. (2003). Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *Journal of Neuroscience Research*, *73*(3), 296–307.
31. Borlongan, C. V., Hadman, M., Sanberg, C. D., & Sanberg, P. R. (2004). Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke*, *35*, 2385–2389.
32. Newman, M. B., Willing, A. E., Manressa, J. J., Sanberg, C. D., & Sanberg, P. R. (2006). Cytokines produced by cultured human umbilical cord blood (HUCB) cells: implications for brain repair. *Experimental Neurology*, *199*(1), 201–208.
33. Vendrame, M., Cassady, J., Newcomb, J., et al. (2004). Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke*, *35*, 2390–2395.
34. Xiao, J., Nan, Z., Motooka, Y., & Low, W. C. (2005). Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury. *Stem Cells Dev.*, *14*, 722–733.
35. Newcomb, J. D., Ajrno, C. T., Sanberg, C. D., et al. (2006). Timing of cord blood treatment after experimental stroke determines therapeutic efficacy. *Cell Transplant*, *15*, 213–223.
36. Vendrame, M., Gemma, C., Pennypacker, K. R., Bickford, P. C., Davis Sanberg, C., Sanberg, P. R., et al. (2006). Cord blood rescues stroke-induced changes in splenocyte phenotype and function. *Experimental Neurology*, *199*(1), 191–200 May.
37. Meier, C., Middelanis, J., Wasielewski, B., Neuhoff, S., Roth-Haerer, A., Gantert, M., et al. (2006). Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatric Research*, *59*(2), 244–249 Feb.

38. Chen, S. H., Chang, F. M., Tsai, Y. C., Huang, K. F., Lin, C. L., & Lin, M. T. (2006). Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heatstroke in the rat. *Experimental Neurology*, *199*(1):67–76, May.
39. Vendrame, M., Gemma, C., de Mesquita, D., Collier, L., Bickford, P. C., Sanberg, C. D., et al. (2005). Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev.*, *14*(5), 595–604 Oct.
40. Nan, Z., Grande, A., Sanberg, C. D., Sanberg, P. R., & Low, W. C. (2005). Infusion of human umbilical cord blood ameliorates neurologic deficits in rats with hemorrhagic brain injury. *Annals of the New York Academy of Sciences*, *1049*, 84–96 May.
41. Nystedt, J., Mäkinen, S., Laine, J., & Jolkkonen, J. (2006). Human cord blood CD34+ cells and behavioral recovery following focal cerebral ischemia in rats. *Acta Neurobiol Exp (Wars).*, *66*(4), 293–300.
42. Mäkinen, S., Kekarainen, T., Nystedt, J., Liimatainen, T., Huhtala, T., Närvänen, A., et al. (2006). Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. *Brain Research*, *1123*(1), 207–215 Dec 6.
43. Chang, C. K., Chang, C. P., Chiu, W. T., & Lin, M. T. (2006). Prevention and repair of circulatory shock and cerebral ischemia/injury by various agents in experimental heatstroke. *Current Medicinal Chemistry*, *13*(26), 3145–54.
44. Bliss, T., Guzman, R., Daadi, M., & Steinberg, G. K. (2007). Cell transplantation therapy for stroke. *Stroke*, *38*, 817–826.
45. Chen, N., Hudson, J. E., Walczak, P., et al. (2005). Human umbilical cord blood progenitors: the potential of these hematopoietic cells to become neural. *Stem Cells*, *23*, 1560–1570.
46. Saporta, S., Kim, J. J., Willing, A. E., et al. (2003). Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. *J. Hematother Stem Cell Res*, *12*, 271–278.
47. Kuh, S. U., Cho, Y. E., Yoon, D. H., et al. (2005). Functional recovery after human umbilical cord blood cells transplantation with brain derived-neurotropic factor into the spinal cord injured rats. *Acta Neurochirurgica (Wein)*, *14*, 985–992.
48. Kang, K. S., Kim, S. W., Oh, Y. H., et al. (2005). Thirty-seven-year old spinal cord-injured female patient, tranplanted of multipotent stem cells from human UC blood with improved sensory perception and mobility, both functionally and morphologically: A case study. *Cytotherapy*, *7*, 368–373.
49. Lu, D., Sanberg, P. R., Mahmood, A., et al. (2002). Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. *Cell Transplant*, *11*, 275–281.
50. Ende, N., & Chen, R. (2002). Parkinson's disease mice and human umbilical cord blood. *J Med*, *33*, 173–80.
51. Gaebuzova-Davis, S., Willing, A. E., Zigova, T., et al. (2003). Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *Journal of Hematotherapy and Stem Cell Research*, *12*, 255–270.
52. Nishio, Y., Koda, M., Kamada, T., Someya, Y., Yoshinaga, K., Okada, S., et al. (2006). The use of hemopoietic stem cells derived from human umbilical cord blood to promote restoration of spinal cord tissue and recovery of hindlimb function in adult rats. *J Neurosurg Spine*, *5*, 424–33.
53. Zhao, Z. M., Li, H. J., Liu, H. Y., Lu, S. H., Yang, R. C., Zhang, Q. J., et al. (2004). Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. *Cell Transplant*, *13*, 113–22.
54. Chen, R., & Ende, N. (2000). The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. *J Med*, *31*, 21–30.
55. Ende, N., Weinstein, F., Chen, R., & Ende, M. (2000). Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). *Life Sciences*, *67*, 53–59.
56. Bachstetter, A. D., Pabon, M. M., Cole, M. J., Hudson, C. E., Sanberg, P. R., Willing, A. E., et al. (2008). Peripheral injection of human umbilical cord blood stimulates neurogenesis in the aged rat brain. 2008. Published online at BMC Neuroscience 9:30; doi: 10.1186/1471–2202–9–22.
57. Nikolic, W. V., Hou, H., Town, T., Zhu, Y., Giunta, B., Sanberg, C. D., Zeng, J., Luo, D., Ehrhart, J., Mori, T., Sanberg Pr, Tan1 J. (2008). Peripherally administered human umbilical cord blood cells reduce parenchymal and vascular beta-amyloid deposits in Alzheimer mice. *Stem Cells Develop.*, *17*, 1–17.