Safety and feasibility of autologous umbilical cord blood transfusion in 2 toddlers with cerebral palsy and the role of low dose granulocyte-colony stimulating factor injections

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Abstract. Purpose: Cerebral palsy (CP) with a prevalence of 2.1 per 1,000 live births generates variable degrees of incurable developmental disability. The aim of the present report was to provide insight in the safety and feasibility of autologous umbilical cord blood (UCB) transfusion with low dose Granulocyte Colony Stimulating Factor (G-CSF) injections in improving the functional outcome of children with cerebral palsy.

Methods: Two toddlers with diagnosed CP were given autologous umbilical cord blood (UCB) transfusion accompanied by low dose subcutaneous granulocyte colony stimulating factor (G-CSF) injections.

Results: Gross Motor Function Classification System (GMFCS) improvements were seen in both without any side effects being noted to date.

Conclusion: In this first report, autologous UCB based intervention in tandem with low dose sc G-CSF administration seems to be feasible and safe with encouraging functional outcome improvements in children with CP.

Keywords: Autologous cord blood stem cell transfusion, cerebral palsy, cord blood, granulocyte-colony stimulating factor, stem cells, umbilical cord, CD34+, Gross Motor Function Classification System, hypoxic-ischemic brain injury, neurogenesis, neuroprotection

1. Introduction

Cerebral palsy (CP) with a prevalence of 2.1 per 1,000 live births (Andersen 2008) is a group of disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of CP often manifesting as hemi, di- or quadriplegia but also as hypertonicity and contractures, are often accompanied by disturbances of sensation, cognition, communication, perception, and/or behavior, and/or a seizure disorder (Bax et al., 2005). The most common causes...
of CP are hypoxic-ischemic brain injury, periventricular leukomalacia or intraventricular and/or parenchymal hemorrhage that occurs in the first year of life (Bax et al., 2005). To date no cure for CP is available and current treatments are targeted to maintaining function, relieving contractures, improving nutrition and providing developmental supportive care and family counseling. Moreover, CP has one of the highest lifetime costs of any congenital disability (921,000 US$) (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5303a4.htm accessed February 2010).

Reports of successful treatments with autologous umbilical cord blood (UCB) have circulated in the media (http://articles.latimes.com/2008/apr/07/health/he-cpalsy7; http://www.foxnews.com/video/index.html?playerId=011008&streamingFormat=FLASH&referralObject=11461523&referralPlaylistId=playlist accessed February 2010) and among the families of affected children and a number of preclinical studies on animal models of cerebral palsy, traumatic brain injury and stroke as well as anecdotal human reports from Duke University have been largely encouraging and without side effects (Harris, 2009). Apart from haematopoietic stem cells, UCB contains other cell populations, such as mesenchymal stem cells, very small embryonic-like stem cells, unrestricted somatic stem cells and endothelial precursor cells all with excellent stem cell capacity and plasticity, characteristics that make autologous UCB a strong candidate for future cell based neurological therapies (Herranz et al., 2009). Autologous human umbilical cord blood stem cells can easily be collected at birth and stored at private or public stem cell banks offering the advantage of sterility and immediate availability for possible future use, if cell therapy is indicated (Herranz et al., 2009). Furthermore, the granulocyte colony stimulating factor (G-CSF) that has extensively been used for over many years in the treatment of neutropenias, oncology and haematopoietic stem cell (HSC) mobilization in numerous populations (including pediatric), both in autologous and allogeneic donation settings, appears safe and without obvious side effects (Welte et al., 1985, Pulsipher, 2006, Tigue, 2007, Cavallaro et al., 2000). Moreover, G-CSF has recently been shown to be a neuronal ligand counteracting programmed cell death and driving neurogenesis (Schneider et al., 2005), characteristics that have been the background for animal and human clinical studies of G-CSF in stroke and traumatic brain and spinal cord injuries (Shyu, 2006, Luo, 2009, Nishio et al., 2007).

The aim of the present study was to provide insight in the safety and feasibility of autologous UCB transfusion with low dose G-CSF injections in improving the functional outcome of children with cerebral palsy.

2. Methods

Two toddlers with previously clinically diagnosed CP by pediatric neurologists causing severe activity limitation with motor signs characteristic of CP like spastic diplegia and toe walking pre and post infusion respectively and without any continuing active neurological or metabolic disease that had stored autologous UCB in our ISO 15189 accredited stem cell laboratory and storage bank emerged requesting release of their samples for transfusion. In both cases cord blood was collected in utero by gravity from an umbilical vein using a sterile personal collection kit including a 21 G needle and a 250 mL labeled blood collection bag containing 35 mL of citrate phosphate dextrose anticoagulant, stored at 15 to 25°C in a validated shipper, and transported to THAI StemLife processing laboratory to be processed immediately after arrival. The process includes red blood cell depletion after Hemohes® addition, plasma reduction and volume depletion after double refrigerated centrifugation. WBC, TNC counts were performed with automated cell counter (Coulter AcT5 diff), as were CD34+ (Coulter flow cytometer Epics-XL), recovery rates and viability (Trypan blue or flow cytometry via Coulter flow cytometer Epics-XL) before post process infectious testing and DMSO addition. Subsequently, after transfer to a Thermogenesis® and overlap bags, controlled rate freezing ensues to −180°C before the final storage in liquid nitrogen at −196°C according to international standards. Written informed parental consent and ethical committee approval (Vejthani Hospital 18-09-2007) were obtained and the subjects were admitted at a hospital of their choice for the transfusion. Infectious disease testing of the autologous UCB sample was negative pre and post storage in both cases. As G-CSF has recently been identified to possess neuroprotective properties (Schneider et al., 2005) and we observed positive motor effects in the index case (case 1) that received it approximately 1 year after the autologous UCB transfusion (Papadopoulos, 2008), we devised a protocol where low-dose G-CSF would be given subcutaneous (sc) prior to the autologous UCB transfusion and that would continue for a certain number of days afterwards in case 2. Our hypothesis (to be applied in our future CP patients) is that a low dose but long duration of G-CSF may offer its postulated neuroprotective advantages similarly to its angio- and vasculogenic effects (Serefanoglu, 2009) without side effects. The dose chosen was 20% of the total dose at 2 µg/day/kg body weight filgrastim sc once daily for 10 days where
the UCB transfusion would occur on the 5th day. On the transfusion day, the subject’s autologous UCB was matched, thawed in a 37°C water bath (post thaw viability 99.5%), and since birth, with a TNC of 508 × 10⁶/ml; CD34+: 0.09%). Ten days after the completion of the intervention (20 after the initiation of the G-CSF), he continued receiving G-CSF for 5 days post transfusion (CD34+: 0.09%). Ten days after the completion of the intervention (20 after the initiation of the G-CSF), he appeared able to stand and move without his orthotic footwear and he gave the impression to be much more stable and mobile as well as physically active during a 2-hour observation in his home environment. Seven months post intervention he is continuously improving and since the 2nd month, he is able to sustain walking more than 30 minutes. He is actively swimming but as yet not running. Hand spasticity has significantly subsided and he is able to hold and manipulate toys with his hands. He is intellectually and physically age congruent. No side effects have been noted to date. He was reclassified as GMFCS level I, up from level III.

Both children were preconditioned with antihistaminics, antiemetics and oxygen prior to the transfusion to fend off eventual DMSO side effects. Mild and transient nausea was noted (< 1 minute) in both. Temperature, BP, SpO2 and HR were all within normal range. The transfusion lasted 30 minutes and the toddlers were discharged after a 6 hour observation.

In MRI scans of the brain and the spinal cord no changes were seen at the time of diagnosis in neither of the two toddlers and one year post transfusion in the 1st one, MRI was normal.

4. Discussion

To the best of our knowledge, this is the first report of an autologous umbilical cord blood based intervention in children with CP and as such an important ob-
neurogenesis and anti-apoptosis, anti-inflammation, angiogenesis and mobilization of bone marrow-derived stem cells to the peripheral blood that migrate over the blood brain barrier to the CNS (24). G-CSF can thus provide its favorable CNS effects via neuroprotection, neurogenesis and angiogenesis by helping penumbral neurons to survive and generating new neural tissue by mobilizing bone marrow and/or endogenous neural stem cells to areas needing reconstruction (Klocke, 2008). G-CSF along with Stem Cell Factor (SCF) have been shown to reduce infarct volume, increase migration and proliferation of stem cells as well as angiogenesis in the injured brain with newly formed blood vessels at the infarct border originating from bone marrow endothelial precursors for up to 2 months post insult (Toth, 2008).

This evidence allows for the hypothesis that G-CSF, used either alone or in combination with another agent, should be an effective strategy in the treatment of cerebral degeneration and spinal cord injuries (Luo, 2009). Ideally, G-CSF could be infused in tandem with autologous umbilical cord blood stem cells to stimulate neurogenesis in the damaged brain (Bachstetter, 2008) aided with its effect in mobilizing bone marrow-derived stem cells to the peripheral blood that migrate over the blood brain barrier to the CNS (Klocke, 2008). Transplanted adult human bone marrow cells have been shown to enter the brain and generate neurons and this phenomenon could be exploited to prevent the development or progression of neurodegenerative diseases or to repair tissue damaged by infarction or trauma (Mezey, 2003). Furthermore, human umbilical cord blood stem cells contain progenitors with the potential to become neural cells, which could then be used in the development of cell-based therapies for brain injuries and diseases (Chen, 2005). Encouraging results have been seen with human umbilical cord blood stem cells in ischemic stroke, amyotrophic lateral sclerosis, brain and spinal cord traumas, Parkinson’s, Huntington’s and Alzheimer’s diseases, opening promising future uses (Herranz, 2009). The mechanism by which human umbilical cord blood stem cells may induce neuroprotection may involves antioxidant(s) and neurotrophic factors, which may, by paracrine and/or autocrine interactions between the insulted cells and the transplanted ones confer neuroprotection (Arien-Zakay, 2009). Moreover, intraperitoneal transplantation of human umbilical cord blood-derived mononuclear cells in a rat model of perinatal brain damage...
lead to both incorporation of these cells in the lesioned brain area and to an alleviation of the neurologic effects of cerebral palsy as assessed by footprint and walking pattern analysis (Meier, 2006).

Hyperbaric oxygen treatment has been assessed in the past in a randomized multicenter trial where the condition of children with CP did not improve compared with slightly pressurized air (Collet, 2001) and similarly we did not notice any further improvement in case 1.

A weakness in our report is that we have no evidence that the administered G-CSF and/or the transfused autologous UCB actually reached the CNS and induced their postulated therapeutic effects. However, the authors, parents and health care providers with pediatric neurology expertise alike observed favorable motor changes such as standing, mobility, swimming and fine motor function improvements, that were improbable to happen within such a short span after the completion of the protocol. In comparison, among children classified as GMFCS level III at age 3, the probability of walking was highest at age 9 at school at 68% (Palisano and Hanna 2009). Moreover, 73% of children (< 6 y old) with CP that were assessed at a mean 4.3 times at 6-month intervals remained in the same level for all GMFCS ratings (Palisano and Cameron, 2006). In contrast, both our two toddlers were reclassified as GMFCS level I within less than 2 months post intervention.

Moreover, the observed initial improvements noted early post infusion can difficultly be attributed to neural regeneration but one reasonable hypothesis may be that the incoming infused autologous UCB CD34+ cells interact with the host parenchymal cells to produce trophic factors that contribute to the prompt functional improvement while G-CSF has been shown to elicit an increased deposition of such CD34+ cells and promote early functional recovery through vascularization/angiogenesis in injured sciatic nerves via neuroprotective functions, including anti-inflammatory, anti-apoptotic, and neurotrophic activity and additional bone marrow stem cell mobilization (Pan, 2009).

In conclusion, in this first report, autologous umbilical cord blood based therapy in tandem with low dose G-CSF administration seems to be feasible and safe with encouraging functional outcome improvements in children with Cerebral Palsy. Additional studies using autologous UCB in the treatment of CP to establish safety and efficacy and further elucidate cell and/or G-CSF dosage and temporal administration, establish of age and GMFCS-appropriate comparative groups are needed, as well as possibly other additions (i.e., Stem Cell Factor, newer stem cell mobilizing agents, and autologous peripheral blood stem cells) in hopes of achieving synergy.

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References


