Neuroprotection by human umbilical cord blood-derived progenitors in ischemic brain injuries

H. ARIEN-ZAKAY[1,2], S. LECHT[1], A. NAGLER[2], P. LAZAROVICI[1]

[1] *The School of Pharmacy Institute for Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel;* [2] *Division of Hematology and Cord Blood Bank, Chaim Sheba Medical Center, Tel-Hashomer, Israel*

ABSTRACT

Stem cells have an extremely high potential to treat many devastating diseases, including neuronal injuries. Albeit the need for human neuronal stem cells, their quantities are very limited by relying on early human embryos as the main source. Therefore, progenitors of other origins, such as human umbilical cord blood (CB) are being considered. In the last decade, various populations isolated from the CB were reported to differentiate in vitro towards a neural phenotype. The conditions to induce the cell differentiation are not conclusive and may include addition of chemicals, cytokines and growth factors, including the nerve growth factor (NGF). Some CB cells were found to express the TrkA-NGF receptor, suggesting an endogenous role for this growth factor also in the CB environment. The ability of CB and derived stem cell populations to protect against neurological deficits was shown, both in vitro and in vivo, in models of ischemic brain injuries. In rodent models of stroke, heatstroke, brain trauma and brain damage at birth, CB cells either by intravenous injection or intrastriatal transplantation, were found to reduce the infarct size and the neurological deficits caused by the injury. The restorative effects of CB were suggested to be mediated by mechanisms other than cell replacement. Some of the proposed mechanisms involve reduced inflammation, nerve fiber reorganization by trophic actions, increased cell survival and enhanced angiogenesis. Furthermore, treatment with CB was found to have a therapeutic window of days compared with the present 3-6 hour window for the treatment of stroke with clinically available tools such as recombinant tissue plasminogen activator. Considering the encouraging results with whole CB and derived cells transplantation in ischemic injury models and since CB is widely available and have been used clinically, they may be an excellent source of cells for treatment of human brain ischemic disorders.

Key words
Human umbilical cord blood • Stemcells • Neuronal • NGF •
Neuroprotection • Ischemia • Brain trauma • Stroke

Ischemic brain injuries: the need for new neuroprotective strategies

Ischemic injury occurs when the blood supply to the tissue is cut off. The length of time a tissue can survive under ischemic insults varies, but eventually all ischemic tissues become apoptotic and/or necrotic (Lipton, 1999). The major pathophysiological effects are caused by the reduction in the oxygen and glucose levels, followed by the renewal of blood supply, responsible for the oxidative damage to the tissue (Mehta et al., 2007). Brain tissue has a complex differential sensitivity towards ischemic insult. Astrocytes can survive from energy deprivation for a prolonged period and are most sensitive to the burst of reactive oxygen species, on the other hand neurons are highly sensitive to oxygen and glucose deprivation (Hertz, 2008). Therefore, global brain ischemia triggers a complex series of biochemical and molecular mechanisms that impairs the neu-

Corresponding Author: Philip Lazarovici, The School of Pharmacy Institute for Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel - Tel.: 972 2 6758 729 - Fax: 972 2-6757 490 - Email: philipl@ekmd.huji.ac.il

rological function through breakdown of cellular integrity and by affecting other elements of brain parenchyma. These mechanisms include excito-toxic glutamatergic hyperactivation, ionic imbalance resulting in calcium overload and generation of pathological levels of free-radicals (Muralikrishna-Adibhatla and Hatcher, 2006).

Cerebral ischemia induced by stroke and brain trauma, causes a generalized death of neuronal tissue within the region affected by the loss of blood flow. On the contrary, neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's, Huntington's and Parkinson's diseases induce cell death of defined neuronal phenotypes in the central nervous system (CNS) (Harris, 2008). In both cases, most therapies for these diseases are rather palliative than restorative and the quality of life of the affected individuals is greatly impaired. First line treatment of brain ischemia is based on thrombolytic therapy, which aims to restore blood flow. However, this treatment does not directly interfere with the ischemic cascade and neuronal damage often continues with restored blood flow. New therapies based on an understanding of the complex interactions of the ischemic cascade may eventually increase the therapeutic window for salvage of the penumbra and provide direct neuroprotection. Combination treatment with a neuroprotective drug or multiple drugs and thrombolytic therapy might theoretically improve ischemic brain outcomes, but this strategy requires confirmation in clinical trials. A major challenge is to develop a neuroprotective therapy for ischemic brain patients to slow, stop, or reverse the progression of the disease. Over the last two decades, more than 500 drugs demonstrated neuroprotective properties in preclinical and clinical setups, however, none have reached the stage of an approved therapy (Zhang et al., 2005; Weinberger, 2006).

Innovative approaches, such as neuroprotection induced by stem cells, raise hope in the clinic. This hope is based on evidences from recent preclinical and clinical studies, which suggest that neural cell-based, regenerative therapies can lead to functional recovery of patients with brain ischemia (Chang et al., 2007). Neural stem cells are multipotent precursors that both selfrenew and give rise to neuronal and glial progenitors (Kennea, and Mehmet, 2002). They are present in the developing (Suter and Krause, 2008) and also in the adult CNS of mammals,

including humans (Nunes et al., 2003; Duan et al., 2008). The ability of neuronal stem cells to differentiate into neuronal or neuron-like cells may replace lost neurons or provide trophic support to tissue at risk in the insulted area of the brain and help to promote survival and neuroprotection (Chang et al., 2007). The major obstacle toward wide use of neuronal stem cells is low numbers of aborted human fetuses from which these cells are isolated and complicated ethical issues that arise. High-quality stem cells with neuroprotective properties, if available in sufficient quantity from other sources, might provide a viable alternative. In this context, human umbilical cord blood (CB) is suggested to contain such cells (Harris, 2008; Arien-Zakay et al., 2010a).

Human umbilical cord blood is an attractive source of cells for brain ischemia therapy

Considering its growing use for hematological reconstitution, its widespread availability and the potential use in non-hematopoietic related-diseases, CB is an attractive source of stem cells for tissue regeneration (Van de Ven et al., 2007; Stanevsky et al., 2009; Arien-Zakay et al., 2010a). With the annual global birth rate of over 100 million per year and the emerging availability of cryopreserving action units, CB is the large underutilized stem cell source with many innate advantages. Due to the naïve nature of a newborn's immune system, a perfect human leukocyte antigen (HLA) match is not required for allogeneic transplantation of CB, which results in fewer acute and chronic graft versus host disease (GVHD) incidences than with bone marrow transplantations (Rocha et al., 2004; Goldstein et al., 2007). The use of CB for transplantations appears to have widespread public support, with 43 public CB banks in 26 countries (Bone Marrow Donors Worldwide, Annual report, 2009). The Israeli public Cord Blood Bank at Sheba Hospital, Tel Hashomer is one of the two European CB banks that received NMDP (bone marrow and stem cell registry in the United States) and AABB (American Association of Blood Banks) accreditation. Therefore the Israeli public CB bank operates in compliance with local and national licensing requirements. Each cord blood unit is tested for the number of nucleated cells, CD34^{+} cell number, colony form-

ing cells (CFU), ABO blood group and Rh type, hemoglobinopathies and bacterial culture, HLA type and certain transmissible infectious diseases. In 2002, the first CB transplantation from a CB unit harvested and frozen at the Tel Hashomer (Sheba) Cord Blood Bank was performed. The CB unit was transplanted in a child with Fanconi's Anemia. Today, the bank has approximately 1,000 cord blood units banked and has released 42 cord blood units for bone marrow transplantation (Nagler, 2009). The successful transplantations of CB as an alternative to bone marrow for the treatment of hematopoietic-related diseases (Stanevsky et al., 2009), provide important evidence that CB-related therapies are feasible and may be further suggested to non-hematopoietic therapies, such as brain injuries.

CB contains cells capable of neuronal differentiation

In the last decade, various subpopulations isolated from CB have been shown to differentiate *in vitro* into neural-like cells and upon administration in animal models of brain ischemia, neurodegenerative diseases and spinal cord injuries they exhibited therapeutic effects (Harris, 2008; Arien-Zakay et al., 2010a). During that time, numerous *in vitro* studies have demonstrated the generation of neuronal cells from CB progenitors (Harris, 2008; Arien-Zakay et al., 2010a). However, the identity of cell type or subpopulation of cells, which is the source of neurons and glia from CB, still remains undefined. Subpopulations of CB isolated according to the expression of hematopoietic stem cells markers such as CD34$^+$ (Bracci-Laudiero et al., 2003), CD133$^+$ (Jang et al., 2004; Zangiacomi et al., 2008) or CD45$^+$ (Rogers et al., 2007) were induced *in vitro* to differentiate towards neuronal-like phenotype. Subsequently, CD34$^-$ CD45$^-$ non-hematopoietic stem cells (Buzanska et al., 2002; Sun et al., 2005; Habich et al., 2006), mesenchymal stem cells (MSC) (Jeong et al., 2004; Lee et al., 2004) and unrestricted somatic stem cells (USSCs) (Kogler et al., 2004, 2006) were identified as origins of the neuronal-like cells.
The experimental approaches to induce *in vitro* neural differentiation also varies, but are mostly based on supplementation of chemical agents, such as retinoic acid (Sanchez-Ramos et al., 2001; Buzanska et al.,

2002; Jang et al., 2004; Habich et al., 2006), dimethylsulfoxide (Jeong et al., 2004) and beta-mercaptoethanol (Ha et al., 2001) as well as of growth factors such as nerve growth factor (NGF) (Arien-Zakay et al., 2007) and interferon-gamma (Arien-Zakay et al., 2009a). The induction of neural phenotypes was characterized by the expression of typical neuronal markers specific to different stages of neural development. These included the expression of neuronal markers such as nestin (Chen et al., 2005a; Arien-Zakay et al., 2007), vimentin (Chen et al., 2005a), NeuN (Arien-Zakay et al., 2009a), MAP-2 (Arien-Zakay et al., 2009a), neurofilaments (Arien-Zakay et al., 2009a), neuronal specific enolase (NSE) (Arien-Zakay et al., 2009a) and the neurotrophin receptors: TrkA (Chen et al., 2005a; Arien-Zakay et al., 2007), TrkB and TrkC (Chen et al., 2005a) as well as the expression of oligodendrocytes and astrocytes markers such as glial fibrilary acidic protein (GFAP) (Rogers et al., 2007). The functionality of CB-derived differentiated neurons was further evaluated by electrical activity measurement, including detection of voltage- and ligand-gated ion channels (Sun et al., 2005). In these cells, gene and partially protein expression for voltage-dependent potassium and sodium channels and the receptors for neurotransmitters acetylcholine (ACh), gamma-aminobutyric acid (GABA), glutamate, glycine, serotonin (5-hydroxytryptamine [5-HT]), and dopamine (DA) were identified and the cells further displayed an inward rectifying potassium current (Kir) and an outward rectifying potassium current (I(K+)) (Sun et al., 2005).

The role of nerve growth factor in CB cells

The group of Dr. L. Aloe was the first to report that the hematopoetic CD34-positive cells in human umbilical CB express NGF and its specific receptor TrkA (Bracci-Laudiero et al., 2003). Our group focused on non-hematopoetic cells and showed NGF responsiveness and neural properties in adherent CB-derived population. Collagen-adherent neuronal progenitors, with positive expression of alpha1 and alpha2 collagen-receptors and the early neuronal marker nestin, were isolated from the mononuclear fraction of CB (Arien-Zakay et al., 2007) (Fig. 1). These cells were capable of differentiating into a neuronal pheno-

Collection of cord blood

I

Isolation of CB-derived neuronal progenitors (HUCBNP)
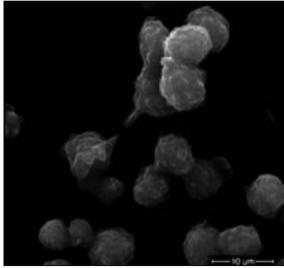
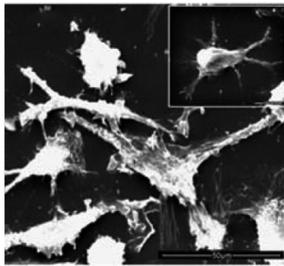Isolation of collagen-adherent cells, expressing α1/2 integrin receptors, 94.8±2.9% nestin-positive.

Cells ID:

Negative for the hematopoietic markers: CD34, CD49c, CD49d, CD62e, CD62p, CD106, CD117, CD133, CD235a, HLA-DRB4, HAS1.

Positive for mesenchymal markers: CD13, CD29, CD44, CD49a,b, CD49e, CD73, CD105, vimentin.

II

NGF and IFN-γ-induced differentiation of HUCBNP

• Expression of long neurite outgrowths

• Induced activation of MAPKs proteins ERK and p38

• Expression of intermediate and mature neuronal mRNA and proteins: nestin, NeuN, β-tubulin III, MAP-2, NSE, NF-M, NF-160, TrkA

III

HUCBNP-induced 30-35% protection against an ischemic injury

• Release of antioxidants

• Correction of the oxidative stress in the injured neuron

• Release of neurotrophic and angiogenic factors (NGF, VEGF, FGF-2)

• Modulation of neurotrophic and angiogenic factors gene expression

Fig. 1 - Chart flow of human umbilical cord blood neuronal progenitors (HUCBNP) isolation, characterization, differentiation and protective properties towards neuronal ischemic damage.

type upon treatment with NGF, interferon-gamma and neuronal conditioning medium. An enhanced differentiation process was observed under supplementation of NGF to interferon-gamma and/or neuronal conditioning medium (Fig. 1). The treated cells exhibited neurite outgrowths of different length and expressed various markers of neuronal cells, at both mRNA and protein level, including nestin, NeuN, MAP-2, neurofilament-160, neurofilament-M, beta-tubulin III and NSE. A small percentage of the treated cells (16%) expressed the glial cells marker, GFAP (Arien-Zakay et al., 2007, 2009a) (Fig. 1). These cells also expressed the NGF receptor TrkA, similar to its expression in CB-derived CD-34$^+$ hematopoietic progenitors (Bracci-Laudiero et al., 2003). In the work of Bracci-Laudiero et al., the researchers showed that hematopoietic stem cells (HSC) and progenitors (CD-34$^+$ cells) present in CB express NGF and its receptor, TrkA. A gradient of TrkA and NGF expression was found to be highest in cord blood CD 34$^+$ cells, reduced in cord blood mononuclear cells (MNC) and minimal in mononuclear cells isolated from adult peripheral blood. These findings suggest that NGF may play a role in the differentiation of hematopoietic progenitors and indicate a different requirement for NGF by immune cells, depending on their state of maturity. The microarray gene expression analysis of the collagen-adherent, neural progenitors, NGF-responsive cells isolated by us indicated that they are negative for the hematopoietic markers CD34, CD49c, CD49d, CD62e, CD62p, CD106, CD117, CD133, CD235a, HLA-DRB4, HLA-DRB4 and HAS1, and positive for the mesenchymal markers CD13, CD29, CD44, CD49a/b, CD49e, CD73, CD105 and vimentin (Arien-Zakay et al., 2009a), supporting their USSCs / MSC origin. Altogether, these results suggest a yet unknown role of NGF and its receptors in both hematopoietic and non-hematopoietic cells within the CB. The results also indicate that the exogenous supplementation of NGF is required in order to induce neuronal differentiation of these progenitors.

CB progenitors confer protection in models of ischemic brain injury

The ability of CB and derived stem cell populations to award protection against neurological deficits was shown *in vivo*, in models of ischemic brain injuries

(Table 1). Using a transient (2-hour) medial carotid artery occlusion (MCAO) focal model of stroke, Chen et al. (2001) were the first to demonstrate that upon CB intravenous administration, many of the physical and behavioral deficits were ameliorated. A significant improvement in functional outcome of motor and modified neurological severity score (mNSS) tests was found in animals given CB cells at 1 day after stroke. At 14 and 35 days after transplantation, intravenously injected CB cells were found in the brain, and significantly more CB cells were found in the ipsilateral damaged hemisphere than in the contralateral control hemisphere. CB cells survived, migrated into the boundary zone of ischemic brain area and some expressed cell type-specific neuronal markers NeuN (2%) and MAP-2 (3%), astrocye marker GFAP (6%) and endothelial cell marker FVIII (8%). *In vitro*, using a brain tissue extract assay, a significant CB cells migration activity was demonstrated in the presence of ischemic cerebral tissue harvested at 24 hours after MCAO compared with the effect of the normal, non-ischemic brain tissue (Chen et al., 2001). Furthermore, using ischemic brain extracts, Newman et al. (2005) suggested that the migration of CB cells into the ischemic area is triggered by cytokines and chemokines released in the ischemic tissue. These observations were further supported by other groups, which monitored the dependence of the beneficial effects on the CB cell dose (Vendrame et al., 2004), mode of cell implantation (Willing et al., 2003) and the timing of transplantation after injury (Newcomb et al., 2006) (Table 1). Using a permanent MCAO, Vendrame et al. (2004) found an inverse relationship between CB cells dose and damaged infarct volume. Furthermore, at 4 weeks after intravenous infusion, there was a significant recovery in behavioral performance only when 10^6 or more CB cells were delivered. Intravenous delivery was suggested to be more effective than striatal delivery in producing long-term (2-months after implantation) functional benefits to the stroke model animals (Willing et al., 2003). The therapeutic efficacy of the treatment was demonstrated even when cells were administrated 48 hr after the injury (Newcomb et al., 2006). Successful treatment at this time point should offer encouragement to clinicians in view that a therapy with a broader window of efficacy may be available to treat ischemic stroke.

Table 1. Neuroprotective effects of CB cells in *in vitro* and *in vivo* ischemic models.

Disease	Ischemic model	Route	CB cell population	Mechanism	Neurological outcome	Reference
Stroke	MCAO	IV	HUCBC	Cells were detected in the brain, some were active for GFAP, NeuN and MAP-2.	Improvement in functional recovery	Chen et al., 2001
		IV (increasing dosage)	HUCBC	Cells were detected in the injured brain hemisphere and in the spleen	Significant improvement in behavioral performance and decrease in infarct volume	Vendrame et al., 2004
		IV	HUCBC	Reduction in the number of CD45$^+$/CD11b$^+$ (microglia/macrophage) and CD45$^+$/B220$^+$(B cell) cells and increase of proinflammatory cytokines such as TNF-α and IL-1β	Increased neuronal survival	Vendrame et al., 2005
		IV	HUCBC	Rescue of neurons in the core, diminished and/or lack of granulocyte and monocyte infiltration and astrocytic and microglial activation in the parenchyma	Both behavioral and physiological recovery	Newcomb et al., 2006
		IV	HUCBC	Rescue of the spleen weight, splenic CD8$^+$ T-cell counts and the amount of brain injury and increasing the production of IL-10 while decreasing IFN-gamma		Vendrame et al., 2006
Stroke	MCAO	IV	nh-UCBSC	Cells were detected in the brain, some positive for NeuN expression. Trophic actions that result in the reorganization of host nerve fiber connections within the injured brain	Improvement in placement and stepping tests and a 50% reduction in lesion volume	Xiao et al., 2005
		IV vs IS	MNC	Long-term (2 months after transplantation) improvement found only after IV delivery	Spontaneous activity decreased indicating behavioral improvement	Willing et al., 2003
		IC	MSC	Cells differentiated into glial, neuronal, and vascular endothelial cells as well as promoted the formation of new vessel angiogenesis	Significantly improvement in neurological function	Ding et al., 2007
		IV	CD34 positive cells	Increased angiogenesis	Enhanced neurogenesis and significant improvement in behavioral tests	Taguchi et al., 2004

Table 1. Neuroprotective effects of CB cells in *in vitro* and *in vivo* ischemic models.

Disease	Ischemic model	Route	CB cell population	Mechanism	Neurological outcome	Reference
	Canine thrombo-embolic brain ischemia	Basilar artery endovascular injection	MSC	Transplanted cells had differentiated into neurons and astrocytes, expressed BDNF and VEGF and were observed in and around endothelial cells that were positive for von Willebrand factor (vWF)	Decrease in the infarction volume, earlier recovery from neurological deficit	Chung et al., 2009
	OGD	*Ex vivo*	HUCBC	Reduction in number of degenerating neurons and production of microglial derived nitric oxide back to levels detected in normoxic controls	Direct protective effects on ischemic brain tissue	Hall et al., 2009
	Tissue extracts after MCAO	*Ex vivo*	MNC	Increased levels of cytokines and chemokines in damaged brain extracts, suggesting their participation in CB cells migration		Newman et al., 2005
Stroke	OGD	*In vitro*	HUCBC	Reduction in number of degenerating neurons and in the production of microglial derived nitric oxide	Significant reductions in brain damage	Hall et al., 2009
	OGD	*In vitro*	Collagen-adherent cells	Secretion of anti-oxidands, reduction in ROS levels in the injured neuron, secretion of NGF, VEGF and FGF and modulation of gene expression.	Reduced neuronal cell death (neuroprotection)	Arien-Zakay et al., 2009
Heatstroke	Ambient temperature (43°C)	IV or IC	HUCBC		Reduction in circulatory shock and cerebral ischemic injury	Chen et al., 2005
TBI	cortical injury	IV	MNC	Cells entered the brain parenchyma and expressed the markers NeuN, MAP-2, GFAP	Reduction of motor and neurological deficits	Lu et al., 2002
Perinatal HIBI	CHID	IP	MNC	Cells entered the brain and were incorporated around the lesion without obvious signs of transdifferentiation	Spastic paresis largely alleviated, resulting in a normal walking behavior	Meier et al., 2006
	CHID	IC	MSC	Transplanted MSC migrated to the hippocampus. MSC differentiated into astrocytes, but not to neurons.	Significant improvement in the neurological severity scores	Xia et al., 2010

Abbreviations: BDNF, brain derived neurotrophic factor; CB, cord blood; CHID, cerebral hypoxic-ischemic damage; FGF-2, basic fibrillary growth factor; HUCBC, human umbilical cord blood cells; IC, intracerebral; IF-gamma, interferon gamma; IL-10, interleukin 10; IP, intraperitoneal; IS, intrastriatal; IV, intravenous injection; MCAO, middle cerebral artery occlusion; MNC, mononuclear cells; MSC, mesenchymal stem cells; NGF, nerve growth factor; nh-UCBSC, nonhematopoietic umbilical cord blood stem cells; ODG, oxygen and glucose deprivation; ROS, reactive oxygen species; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor.

In vivo animal studies succeeded in using CB also for the treatment of heat stroke (Table 1). Under an exposure to an ambient temperature of 43 ℃, rats transplanted with intravenous or intracerebroventricular CB cells showed a significant improvement in their survival as compared to the non-transplanted group (61-148 min. vs. 21-23 min., respectively) (Chen et al., 2005b). The circulatory shock, intracranial hypertension, cerebral hypoperfusion and hypoxia, increment of cerebral ischemia, and damage markers during heat stroke were all significantly attenuated by the delivery of CB cells but not peripheral blood mononuclear cells. Furthermore, treatment with CB-derived CD34+ cells significantly improved survival time (63-291 min) while causing attenuation of hypotension, hepatic and renal failure, hypercoagulable state, inflammation, cerebral ischemia and injury heatstroke reactions. In addition, the levels of IL-10 in plasma and glial cell line-derived neurotrophic factors (GDNF) in brain were all significantly increased after CB-CD34+ cell therapy during heatstroke (Chen et al., 2007). This data indicate that CB, but not peripheral blood, cell therapy may resuscitate persons who had a heatstroke by reducing multiorgan dysfunction or failure.

Traumatic brain injury is another potential target for treatment using CB stem cells as was first indicated by Lu et al. (2002) (Table 1). They observed homing of intravenous transplanted cells into the parenchyma of brain lesion and a decrease in neurological damage in a rat model. The cells expressed the neuronal markers, NeuN and MAP-2, and the astrocytic marker, GFAP. Some CB cells integrated into the vascular walls within the boundary zone of the injured area (Lu et al., 2002).

Treatment with CB cells was also proposed for other brain damages, such as ischemic damage at birth (Table 1). Using brain damaged neonatal rats, Meier et al. observed both incorporation of CB mononuclear cells in the lesioned brain area and an alleviation of the neurological effects of cerebral palsy as assessed by footprint and walking pattern analysis (Meier et al., 2006). This was also observed under intracerebral transplantation of CB-derived MSCs, which were found to differentiate into astrocytes, but not neurons (Xia et al., 2010). Furthermore, a pilot study is in progress at Duke University to test the feasibility (of collection, preparation and infusion) using autologous CB on a baby born

with signs of brain injury during the first 14 days after birth (http://www.clinicaltrials.gov/ct2/show/NCT00593242?order =1).

CB-derived cells for treatment of ischemic brain injury: mechanistic aspects

Although functional improvement and reductions in lesion volume were observed in ischemic rodents treated with CB cells, cells expressing the human nuclei marker within the rodent brain were rather scant, suggesting that the restorative effects of CB may be mediated by mechanisms other than cell replacement (Xiao et al., 2005) (Table 1). Some of the mechanisms proposed involved reduced inflammation (Newcomb et al., 2006; Vendrame et al., 2006; Hall et al., 2009), nerve fiber reorganization by trophic actions (Xiao et al., 2005), increased cell survival and enhanced angiogenesis (Ding et al., 2007; Taguchi et al., 2004) (Table 1). Evidences for reduced inflammation included the suppression of lymphocytes (Hall et al., 2009), granulocyte and monocyte infiltration (Newcomb et al., 2006) and the lack of astrocytic and microglial activation in the parenchyma (Newcomb et al., 2006). The evidences for reduced inflammation also included the rescue of spleen weight and splenic CD8+ T-cell counts and an increase in the production of IL-10 while decreasing IFN-gamma (Vendrame et al., 2006). To determine whether CB cells could exert trophic effects on the host brain, Xiao et al. (2005) directly transplanted the cells into the brain parenchyma after ischemic brain injury and showed an increased sprouting of nerve fibers from the non damaged hemisphere into the ischemia-damaged side of the brain. Their results suggest that restorative effects observed with CB cells treatment following ischemic brain injury may be mediated by trophic actions that results in the reorganization of host nerve fiber connections within the injured brain. CB-MCSs (Ding et al., 2007) and CB-CD34 positive cells (Taguchi et al., 2004) were suggested to promote either directly or indirectly an environment permissive to neovascularization of ischemic brain so that neuronal regeneration can proceed. Ding et al. (2007) showed that transplanted CB-MSCs migrated towards the ischemic boundary zone, were able to differentiate into

glial, neuronal, doublecortin[+], CXCR4^{+}, and vascular endothelial cells and that CB cells transplantation promoted the formation of new blood vessels. These results suggest an enhanced neuroplasticity as well as an increase local cortical blood flow in the ischemic hemisphere, supporting brain plasticity and recovery. Furthermore, in a canine thromboembolic brain ischemia model, transplanted CB-MSCs had differentiated into neurons and astrocytes and were observed in and around endothelial cells that were positive for von Willebrand factor (vWF) (Chung et al., 2009). These cells expressed neuroprotective factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), at 4 weeks after the transplantation, in correlation to reduced infarct volume and earlier recovery from the neurological deficit (Chung et al., 2009) (Table 1).

We and others have widely explored these mechanisms *in vitro* (Arien-Zakay et al., 2009b; Vendrame et al., 2005; Hau et al., 2008) (Table 1). CB-derived progenitors were shown to confer neuroprotection in models of cerebral ischemia using oxygen glucose deprivation (OGD) insulted adrenal medulla neurons (Arien-Zakay et al., 2009b), primary rat cortical neuronal cultures exposed to hypoxia and hippocampal slice cultures exposed to OGD (Hall et al., 2009; Vendrame et al., 2005), as well as on differentiated neuroblastoma SH-SY5Y cells exposed to hypoxia (Hau et al., 2008). This protection is explained by a mechanism involving the release of antioxidants, the decrease in numbers of free radicals in the injured neuron and the local accumulation of growth factors in the media. This includes the release of NGF, VEGF and basic fibroblast growth factor (FGF-2) and the modulations of neurotrophic and angiogenic factors gene expression (Arien-Zakay et al., 2009b). As these CB cells selectively home into the lesioned brain areas (Chen et al., 2001; Lu et al., 2002; Newman et al., 2005; Meier et al., 2006; Ding et al., 2007), the release of neurotrophic and angiogenic factors (Cho et al., 2006; Alexanian et al., 2008) and/or antioxidants *in vivo*, as shown *in vitro* (Arien-Zakay et al., 2009b), would be highly specific and reaching local therapeutic concentration in damaged brain area, therefore supporting a "bystander" neuroprotective strategy (Martino and Pluchino, 2006). This strategy refers to an effect mediated by soluble growth factors and not by neurogenesis (proliferation and differentiation of neuronal stem cells replacing neuronal network). Therefore, the concept of bystander cell therapy, which involves the implant/transplant of neuronal stem cells with the ability to secrete neurotrophins in the insulted area, is suggested to replace or complement the common method of providing neurotrophins to the injured brain (Castrén, 2004).

Considering the encouraging preclinical and clinical trials, reporting improvement of neurological deficits together with accumulating data on the differentiation of CB-derived populations into a variety of neuronal phenotypes, it may be realistic that CB will become an important factor for treatment of neurological illnesses.

Conclusion

Although not yet clinically available for CNS disorders, stem cells technology is expected to evolve into a powerful tool in therapy of brain ischemia (Arien-Zackay 2010b). CB-derived cells offer multiple advantages over adult stem cells and embryonic stem cells, including their immaturity, which may play a significant role in the rejection of generated tissue when transplanted into a mismatched host, their simple collection and their ethically acceptability for transplantations in humans.

During the last decade, a growing body of evidence suggests that CB contains cells capable of differentiating into neural phenotypes, including neurons, astroglia and oligodendroglia. Currently, the cells differentiation towards a neural phenotype involves various protocols and the neuroprotective properties of the isolated cells according to their hematological phenotype is not clear. Furthermore, certain growth factors, including NGF, which were found to involve in neural differentiation of CB cells grown in vitro, may also have some yet undefined roles in the CB environment, suggested to be related to immune cell differentiation (Bracci-Laudiero et al., 2003).

CB-derived cell populations are now under investigation to determine their possible application for treatment of neurological diseases. Animal models have shown that CB-cells improve recovery in neurodegenerative diseases animal models such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer's diseases (Harris, 2008, Arien-Zakay

et al., 2010a). CB cells were also widely shown to enhance animal recovery after ischemic brain injuries such as stroke, heatstroke, traumatic brain injury and hypoxic-ischemic damage around birth. It is important to note however, that the cell therapy strategy may differ according to the pathophysiology of the disease. Whereas in chronic neurodegenerative diseases a defined neuronal pathway is degenerated, in acute ischemic brain injuries the damage occurs within a whole region of the brain including neurons, glia and blood capillaries. A rational treatment for ischemic brain injuries will therefore include the support of the injured cell survival (bystander effect) and an induction of endogenous neurogenesis, while for the treatment of neurodegenerative diseases, a cell replacement approach may be more effective. Indeed, upon CB cells transplantation in brain ischemia-damaged animals, the infarct area was decreased in a reverse correlation to the transplanted CB cells number and a significant improvement in functional outcome in neurological deficits was observed. Several mechanisms of action were suggested including reduction in inflammatory response, reorganization of nerve fiber by trophic actions, rescue of damaged cells and enhancement of angiogenesis. These mechanisms fit the bystander approach suggested to be more suitable for the treatment of ischemic insult. Furthermore, both intravenous and striatal delivery were suggested to be effective in producing long-term functional benefits to the ischemic brain injury animals and the therapeutic efficacy of the treatment was demonstrated even when cells were administrated 48 hr after the injury. Since ischemic brain injuries are known for the narrow time-window for current available therapeutic activity is possible, the administration of CB cells beyond the hyperacute phase of ischemia may amplify the intrinsic properties of the brain regarding neuroplasticity and subsequent neurological recovery.

Therefore, although still in the preclinical stage of research, treatment by CB cells, which may extend the therapeutic time-window and provides significant improvements in neurological deficits, holds a tremendous potential for therapy and may create an opportunity to treat most, if not all, ischemic brain patients. For this aim, the next decade dedicated to comprehensive research of the therapeutic properties of CB for ischemic brain treatment will provide

stronger rationale for considering initiation of clinical studies in humans. This will include determination of the identity of the cells active in neurorecovery and the cell populations of their origin, the mechanisms of therapeutic effects, the route of cell administration, the time window for intervention and others.

Acknowledgment

References

Alexanian A.R., Maiman D.J., Kurpad S.N., Gennarelli T.A. *In vitro* and *in vivo* characterization of neurally modified mesenchymal stem cells induced by epigenetic modifiers and neural stem cell environment. *Stem Cells Dev.*, **17**: 1123-1130, 2008.

Arien-Zakay H., Nagler A., Galski H., Lazarovici P. Neuronal conditioning medium and nerve growth factor induce neuronal differentiation of collagen-adherent progenitors derived from human umbilical cord blood. *J. Mol. Neurosci.*, **32**: 179-191, 2007.

Arien-Zakay H., Lecht S., Bercu M.M., Amariglio N., Rechavi G., Galski H., Lazarovici P., Nagler A. Interferon-gamma-induced neuronal differentiation of human umbilical cord blood-derived progenitors. *Leukemia*, **23**: 1790-1800, 2009a.

Arien-Zakay H., Lecht S., Bercu M.M., Tabakman R., Kohen R., Galski H., Nagler A., Lazarovici P. Neuroprotection by cord blood neural progenitors involves antioxidants, neurotrophic and angiogenic factors. *Exp. Neurol.*, **216**: 83-94, 2009b.

Arien-Zakay H., Lazarovici P., Nagler A. Tissue regeneration potential in human umbilical cord blood. *Best. Pract. Res. Clin. Haematol.*, **23**: 291-303, 2010a.

Arien-Zakay H., Lecht S., Nagler A., Lazarovici P. Human umbilical cord blood stem cells: rational for use as a neuroprotectant in ischemic brain disease. *Int. J. Mol. Sci.*, **11**: 3513-3528, 2010b.

Bone Marrow Donors Worldwide, Annual report, 2009, http://www.bmdw.org/uploads/media/BMDW2009.pdf

Bracci-Laudiero L., Celestino D., Starace G., Antonelli A., Lambiase A., Procoli A., Rumi C.,

Lai M., Picardi A., Ballatore G., Bonini S., Aloe L. CD34-positive cells in human umbilical cord blood express nerve growth factor and its specific receptor TrkA. *J Neuroimmunol.*, **136**: 130-139, 2003.

Buzanska L., Machaj E.K., Zablocka B., Pojda Z., Domanska-Janik K. Human cord blood-derived cells attain neuronal and glial features in vitro. *J. Cell Sci.*, **115**: 2131-2138, 2002.

Castrén E. Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection. *Mol. Neurobiol.*, **29**: 289-301, 2004.

Chang Y.C., Shyu W.C., Lin S.Z., Li H. Regenerative therapy for stroke. *Cell Transplant.*, **16**: 171-181, 2007.

Chen J., Sanberg P.R., Li Y., Wang L., Lu M., Willing A.E., Sanchez-Ramos J., Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke*, **32**: 2682-2688, 2001.

Chen N., Hudson J.E., Walczak P., Misiuta I., Garbuzova-Davis S., Jiang L., Sanchez-Ramos J., Sanberg P.R., Zigova T., Willing A.E. Human umbilical cord blood progenitors: the potential of these hematopoietic cells to become neural. *Stem Cells*, **23**: 1560-1570, 2005a.

Chen S.H., Chang F.M., Tsai Y.C., Huang K.F., Lin C.L., Lin M.T. Resuscitation from experimental heatstroke by transplantation of human umbilical cord blood cells. *Crit. Care Med.*, **33**: 1377-1383, 2005b.

Chen S.H., Chang F.M., Chang H.K., Chen W.C., Huang K.F., Lin M.T. Human umbilical cord blood-derived CD34+ cells cause attenuation of multiorgan dysfunction during experimental heat-stroke. *Shock*, **27**: 663-671, 2007.

Cho S.W., Gwak S.J., Kang S.W., Bhang S.H., Won Song K.W., Yang Y.S., Choi C.Y., Kim B.S. Enhancement of angiogenic efficacy of human cord blood cell transplantation. *Tissue Eng.*, **12**: 1651-1661, 2006.

Chung D.J., Choi C.B., Lee S.H., Kang E.H., Lee J.H., Hwang S.H., Han H., Lee J.H., Choe B.Y., Lee S.Y., Kim H.Y. Intraarterially delivered human umbilical cord blood-derived mesenchymal stem cells in canine cerebral ischemia. *J. Neurosci. Res.*, **87**: 3554-3567, 2009.

Ding D.C., Shyu W.C., Chiang M.F., Lin S.Z., Chang Y.C., Wang H.J., Su C.Y., Li H. Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. *Neurobiol. Dis.*, **27**: 339-353, 2007.

Duan X., Kang E., Liu C.Y., Ming G.L., Song H. Development of neural stem cell in the adult brain. *Curr. Opin. Neurobiol.*, **18**: 108-115, 2008.

Goldstein G., Toren A., Nagler A. Transplantation and other uses of human umbilical cord blood and stem cells. *Curr. Pharm. Des.*, **13**: 1363-1373, 2007.

Habich A., Jurga M., Markiewicz I., Lukomska B., Bany-Laszewicz U., Domanska-Janik K. Early appearance of stem/progenitor cells with neural-like characteristics in human cord blood mono-nuclear fraction cultured *in vitro*. *Exp. Hematol.*, **34**: 914-925, 2006.

Ha Y., Choi J.U., Yoon D.H., Yeon D.S., Lee J.J., Kim H.O., Cho Y.E. Neural phenotype expression of cultured human cord blood cells *in vitro*. *Neuroreport*, **12**: 3523-3527, 2001.

Hall A.A., Leonardo C.C., Collier L.A., Rowe D.D., Willing A.E., Pennypacker K.R. Delayed treatments for stroke influence neuronal death in rat organotypic slice cultures subjected to oxygen glucose deprivation. *Neuroscience*, **164**: 470-477, 2009.

Harris D.T. Cord blood stem cells: a review of potential neurological applications. *Stem Cell Rev.*, **4**: 269-274, 2008.

Hau S., Reich D.M., Scholz M., Naumann W., Emmrich F., Kamprad M., Boltze J. Evidence for neuroprotective properties of human umbilical cord blood cells after neuronal hypoxia in vitro. *BMC Neurosci.*, **9**: 30, 20008.

Hertz L. Bioenergetics of cerebral ischemia: a cellular perspective. *Neuropharmacology*, **55**: 289-309, 2008.

Http://www.clinicaltrials.gov/ct2/show/NCT00593242?order =1

Jang Y.K., Park J.J., Lee M.C., Yoon B.H., Yang Y.S., Yang S.E., Kim S.U. Retinoic acid-mediated induction of neurons and glial cells from human umbilical cord-derived hematopoietic stem cells. *J. Neurosci. Res.*, **75**: 573-584, 2004.

Jeong J.A., Gang E.J., Hong S.H., Hwang S.H., Kim S.W., Yang I.H., Ahn C., Han H., Kim H. Rapid neural differentiation of human cord blood-derived mesenchymal stem cells. *Neuroreport*, **15**: 1731-1734, 2004.

Kennea N.L. and Mehmet H. Neural stem cells. *J. Pathol.*, **197**: 536-550, 2002.

Kogler G., Sensken S., Airey J.A., Trapp T., Müschen M., Feldhahn N., Liedtke S., Sorg R.V., Fischer J., Rosenbaum C.; Greschat S., Knipper A., Bender

J., Degistirici O., Gao J., Caplan A.I., Colletti EJ, Almeida-Porada G., Müller H.W., Zanjani E., Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J. Exp. Med.*, **200**: 123-135, 2004.

Kogler G., Sensken S., Wernet P. Comparative generation and characterization of pluripotent unrestricted somatic stem cells with mesenchymal stem cells from human cord blood. *Exp. Hematol.*, **34**: 1589-1595, 2006.

Lee O.K., Kuo T.K., Chen W.M., Lee K.D., Hsieh S.L., Chen T.H. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood*, **103**: 1669-1675, 2004.

Lipton P. Ischemic cell death in brain neurons. *Physiol Rev.*, **79**: 1431-1568, 1999.

Lu D., Sanberg P.R., Mahmood A., Li Y., Wang L., Sanchez-Ramos J., Chopp M. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. *Cell Transplant.*, **11**: 275-281, 2002.

Martino G. and Pluchino S. The therapeutic potential of neural stem cells. *Nat. Rev. Neurosci.*, **7**: 395-406, 2006.

Mehta S.L., Manhas N., Raghubir R. Molecular targets in cerebral ischemia for developing novel therapeutics. *Brain Res. Rev.*, **54**: 34-66, 2007.

Meier C., Middelanis J., Wasielewski B., Neuhoff S., Roth-Haerer A., Gantert M., Dinse H.R., Dermietzel R., Jensen A. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr. Res.*, **59**: 244-249, 2006.

Muralikrishna-Adibhatla R. and Hatcher J.F. Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic. Biol. Med.*, **40**: 376-387, 2006.

Nagler A. Recent developments in human umbilical cord blood transplantation in Israel. *Biol. Blood Marrow Transplant.*, **15**: 99-103, 2009.

Newcomb J.D., Ajrno C.T., Sanberg C.D., Sanberg P.R., Pennypacker K.R., Willing A.E. Timing of cord blood treatment after experimental stroke determines therapeutic efficacy. *Cell Transplant.*, **15**: 213-223, 2006.

Newman M.B., Willing A.E., Manresa J.J., Davis-Sanberg C., Sanberg P.R. Stroke-induced migration of human umbilical cord blood cells: time course and cytokines. *Stem Cells Dev.*, **14**: 576-586, 2005.

Nunes M.C., Roy N.S., Keyoung H.M., Goodman R.R., McKhann G. 2nd., Jiang L., Kang J., Nedergaard M., Goldman S.A. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat. Med.*, **9**: 439-447, 2003.

Rocha V., Labopin M., Sanz G., Arcese W., Schwerdtfeger R., Bosi A., Jacobsen N., Ruutu T., de Lima M., Finke J., Frassoni F., Gluckman E.; Acute Leukemia Working Party of European Blood and Marrow Transplant Group; Eurocord-Netcord Registry. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N. Engl. J. Med.*, **351**: 2276-2285, 2004.

Rogers I., Yamanaka N., Bielecki R., Wong C.J., Chua S., Yuen S., Casper R.F. Identification and analysis of in vitro cultured CD45-positive cells capable of multi-lineage differentiation. *Exp. Cell. Res.*, **313**: 1839-1852, 2007.

Sanchez-Ramos J.R., Song S., Kamath S.G., Zigova T., Willing A., Cardozo-Pelaez F., Stedeford T., Chopp M., Sanberg P.R. Expression of neural markers in human umbilical cord blood. *Exp. Neurol.*, **171**: 109-115, 2001.

Stanevsky A., Goldstein G., Nagler A. Umbilical cord blood transplantation: pros, cons and beyond. *Blood Rev.*, **23**: 199-204, 2009.

Sun W., Buzanska L., Domanska-Janik K., Salvi R.J., Stachowiak M.K. Voltage-sensitive and ligand-gated channels in differentiating neural stem-like cells derived from the nonhematopoietic fraction of human umbilical cord blood. *Stem Cells*, **23**: 931-945, 2005.

Suter D.M. and Krause K.H. Neural commitment of embryonic stem cells: molecules, pathways and potential for cell therapy. *J. Pathol.*, **215**: 355-368, 2008.

Taguchi A., Soma T., Tanaka H., Kanda T., Nishimura H., Yoshikawa H., Tsukamoto Y., Iso H., Fujimori Y., Stern D.M., Naritomi H., Matsuyama T. Administration of CD34 cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J. Clin. Invest.*, **114**: 330-338, 2004.

Van de Ven C., Collins D., Bradley M.B., Morris E., Cairo M.S. The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration. *Exp. Hematol.*, **35**: 1753-1765, 2007.

Vendrame M., Cassady J., Newcomb J., Butler T., Pennypacker K.R., Zigova T., Sanberg C.D., Sanberg P.R., Willing A.E. Infusion of human

umbilical cord blood cells in a rat model of stroke dosedependently rescues behavioral deficits and reduces infarct volume. *Stroke*, **35**: 2390-2395, 2004.

Vendrame M., Gemma C., de Mesquita D., Collier L., Bickford P. C., Sanberg C. D., Sanberg P. R., PennyInvest packer K. R., Willing A. E. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev.*, **14**: 595-604, 2005.

Vendrame M., Gemma C., Pennypacker K.R., Bickford P.C., Davis Sanberg C., Sanberg P.R., Willing A.E. Cord blood rescues stroke-induced changes in splenocyte phenotype and function. *Exp. Neurol.*, **199**: 191-200, 2006.

Weinberger J.M. Evolving therapeutic approaches to treating acute ischemic stroke. *J. Neurol. Sci.*, **249**: 101-109, 2006.

Willing A.E., Lixian J., Milliken M., Poulos S., Zigova T., Song S., Hart C., Sanchez-Ramos J., Sanberg P.R. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J. Neurosci. Res.*, **73**: 296-307, 2003.

Xia G., Hong X., Chen X., Lan F., Zhang G., Liao L. Intracerebral transplantation of mesenchymal stem cells derived from human umbilical cord blood alleviates hypoxic ischemic brain injury in rat neonates. *J. Perinat. Med.*, **38**: 215-221, 2010.

Xiao J., Nan Z., Motooka Y., Low W.C. Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neu-rological deficits associated with ischemic brain injury. *Stem Cells Dev.*, **14**: 722-733, 2005.

Zangiacomi V., Balon N., Maddens S., Lapierre V., Tiberghien P., Schlichter R., Versaux-Botteri C., Deschaseaux F. Cord blood-derived neurons are originated from CD133^{+}/CD34 stem/progenitor cells in a cell-to-cell contact dependent manner. *Stem Cells Dev.*, **17**: 1005-1016, 2008.

Zhang R.L., Zhang Z.G., Chopp M. Neurogenesis in the adult ischemic brain: generation, migration, survival, and restorative therapy. *Neuroscientist*, **11**: 408-416, 2005.