Impact of Neonatal Asphyxia on Stem Cell Viability and the Effect of Cerebrolysin on its Revitalization

Thesis
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Abstract

Perinatal fetal distress with subsequent hypoxic ischemic encephalopathy is the leading cause for brain injury and mortality in neonates worldwide. The neuron-regenerative potential of cord blood stem cells has been proven in many experimental animal models. Hypoxia induces mobilization and activation of hemopoietic and mesenchymal stem cells. Umbilical cord is a rich source for mesenchymal stem cells that can be a potential source for autologous transplantation for tissue regeneration. This work is designed to examine the viability, cycling state and neural transdifferentiation potential of cord blood mononuclear cells in hypoxic, normoxic and preterm cord blood.

Key Words: Hypoxia– Stem cells –Neural Transdifferentiation.

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AIF: apoptosis inducing factor

ALS: amyotrophic lateral sclerosis

AMPA: -alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

Apaf-1: cofactor caspase-9 cofactor protein

ASC: adult stem cell

ATP: adenosine triphosphate

BE: base excess

bFGF: basic fibroblast growth factor

BMSCs: bone marrow stromal cell

BM-MSC: bone marrow mesenchymal stem cell

BSA: bovine serum albumin

CFC: colony-forming cells

CKBB: creatine kinase brain-type

CNS: central nervous system

CO2: carbon dioxide

CP: cerebral palsy

CPAP: continuous positive airway pressure

CS: caesarian section

CT: computed tomography

Cyt C = cytochrome C

DD: death domain

DED: death effector domain

DM: diabetes mellitus

DNA: deoxyribo nucleic acid

DTI: diffusion tensor imaging

EEG: electroencephalography

EGF: epidermal growth factor

EPO: erythropoietin

eNOS: endothelial nitric oxide synthase

FA: fractional anisotropy

FGF: fibroblast growth factor

FHR: fetal heart rate

FT: full term

G: gram

GA: gestational age

GFAP: Glial-Fibrillary-Acidic-Protein

Gr.: group

GVHD: graft-versus host disease

hESC: Human embryonic stem cells

HI: hypoxia ischemia

HIE: hypoxic-ischemic encephalopathy

HPC: human progenitor cell

HSC: human stem cells

HSPC: human stem progenitor cell

HTN: hypertension

HUCB: human umbilical cord blood

IAP: inhibitor of apoptosis protein

ICAD: inhibitor of caspase activated DNAase

ICM: inner cell mass

IL: interleukin

IVF: in vitro fertilization

iNOS: inducible nitric oxide synthase

IVF: in vitro fertilization

IVH: intraventricular hemorrhage

MAP-2: microtubuli associated protein-2

MAS: meconium aspiration syndrome

MIF: macrophage inflammatory protein

MCAO: middle cerebral artery occlusion

MNCs: mononuclear cells

MRI magnetic resonant imaging

MSC: mesenchymal stem cells

NE: Neonatal encephalopathy

NGF: nerve growth factor

NF-I: anti neuron specific tubulin.

NMDA: N-methyl-D-aspartate

nNOS: neuronal nitric oxide synthase

NO: nitric oxide

NPO: nothing per mouth

NSC: neural stem cells

O2: oxygen

OFR: oxygen free radicals

PBS: phosphate buffered saline

PBSC: peripheral blood stem cells

PCB: preterm cord blood

PDA: patent ductus arteriosus

PROM: premature rupture of membranes

PT: preterm

PVH: periventricular hemorrhage

PVL: periventricular leukomalacia

PVWM: Periventricular white-matter

SVZ: subventricular zone

TCB: term cord blood

TNC: total nucleated cells

TNF: tumor necrosis factor

UCB: umbilical cord blood

UCBT: umbilical cord blood transplantation

US: United States

VD: vaginal delivery

VEGF: vascular endothelial growth factor

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Introduction and Aim of Work

Introduction

Hypoxic ischemic encephalopathy is often one of the most devastating sequelae encountered in the newborn period. Although the predominant injury affects the brain, almost every organ system in the body is negatively impacted. Cerebral palsy, seizure activity, and varying degrees of developmental delays are some of the chronic disabilities seen in survivors, (*Verklan 2009).*

Term and preterm infants sustain different types of injury. The type and severity of brain damage sustained by the term infant is modulated by infection, extended labor or repeated asphyxia after birth. The damage sustained by the preterm infant has been classically referred to as periventricular leukomalacia (PVL), which is defined as focal and diffuse damage to the periventricular white matter.

Stem cells possess the ability for unlimited or prolonged self-renewal and to differentiate into highly distinct cell lineages which makes them attractive for a wide range of clinical and pharmacological applications (*Klimanskaya et al., 2008*). HUCB contains multiple populations of pluripotent stem cells and can be considered an alternative to embryonic stem cells.

Interestingly, sub-populations of HUCB cells, either hematopoietic or mesenchymal-like cells upon treatment with specific growth factors are able to differentiate into neuron-like cells in culture (*Sun et al., 2005),* and thus amenable to treatment of neurologic diseases (*Harris et al., 2008*). UCB contains a high percentage of CD34+ and CD105+ cells (markers of stemness), implying outstanding regenerative potential, (*Verneris M.R. and Miller, 2009).*

Cerebrolysin consists of low molecular weight peptides with neuroprotective and neurotrophic properties similar to naturally occurring growth factors supporting the survival, stability, and function of neurons. Cerebrolysin decreases amyloid production, promotes synaptic repair, and improves cognitive and behavioral performance. The effects of Cerebrolysin have been investigated and confirmed in various cell culture and animal models of neurodegeneration and ischemia.

Given hypoxic conditions are the physiologic norms for a variety of stem cell niches, more research has incorporated hypoxia into tissue culture technique. A variety of studies demonstrate significant benefit in terms of cell proliferation using low oxygen tensions, (*Studer et al., 2000).* Hypoxia may be used as a stimulus to promote differentiation into various cell lines. Recent reports have shown that hypoxia can regulate the proliferation and differentiation of stem cells, and that, especially, mild hypoxia has salutary effects on stem／progenitor cells, (*Zhu et al., 2005).*

The influence of invivo hypoxia on the proliferation and neural differentiation of UCB stem cells was evaluated in this work. Cord blood samples from potentially asphyxiated FT neonates, healthy FT neonates and preterm neonates were collected and subjected to neural differentiation in culture media containing cerebrolysin.

Aim of work

The aim of the present study is to explore stem cell viability and proliferation in asphyxiated neonates and find out:

- Whether multipotent mesenchymal cells particularily with a neurogenic potential can be obtained from umbilical cord of asphyxiated newborn and if these cells are viable and have higher neural differentiation potential than stem cells from normal neonates, therefore can be used for early rescue of their injured brain.
- The effect of hypoxia on stem cell number and stem cell subsets, (CD34; marker of stemness, CD38; marker of heamatopoeitic commitment).
- Whether Cerebrolysin can enhance the neurogenic differentiation of stem cells in vitro.
- This study will also explore the quality of preterm cord blood regarding stem cell number, stem cell subset, neural differentiation potential and proliferative capacity.