Cell Transplantation
2019, Vol. 28(5) 497–509

DOI: 10.1177/0963689718809658
journals.sagepub.com/home/cll
SAGE

A New Approach to Cerebral Palsy Treatment: Discussion of the Effective Components of Umbilical Cord Blood and its Mechanisms of Action

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Abstract

Cerebral palsy (CP) includes a group of persistent non-progressive disorders affecting movement, muscle tone, and/or posture. The total economic loss during the life-span of an individual with CP places a heavy financial burden on such patients and their families worldwide; however, a complete cure is still lacking. Umbilical cord blood (UCB)-based interventions are emerging as a scientifically plausible treatment and possible cure for CP. Stem cells have been used in many experimental CP animal models and achieved good results. Compared with other types of stem cells, those from UCB have advantages in terms of treatment safety and efficacy, ethics, non-neoplastic proliferation, accessibility, ease of preservation, and regulation of immune responses, based on findings in animal models and clinical trials. Currently, the use of UCB-based interventions for CP is limited as the components of UCB are complex and possess different therapeutic mechanisms. These can be categorized by three aspects: homing and neuroregeneration, trophic factor secretion, and neuroprotective effects. Our review summarizes the features of active components of UCB and their therapeutic mechanism of action. This review highlights current research findings and clinical evidence regarding UCB that contribute to treatment suggestions, inform decision-making for therapeutic interventions, and help to direct future research.

Keywords

umbilical cord blood, cerebral palsy, stem cells, cell transplantation, mechanism

Introduction

Cerebral palsy (CP) is a group of persistent disorders caused by brain injury during prenatal or postnatal periods. CP affects movement, muscle tone, and/or posture, and results from non-progressive disturbances of the developing central nervous system (CNS)[1]. The earliest description of the disorder is attributed to the orthopedic surgeon William Little in 1862[2]. The prevalence of CP in countries with advanced medical care is 2.22–2.90 per 1000, and is likely higher in economically disadvantaged locations[3]. Generally, hypoxia-induced brain damage, genetic factors, mutations, and several other hypothesized theories including infection/inflammation could lead to hypoplastic brain tissue, resulting in loss of neuron function[4]. CP can be divided into five types based on motor dysfunction (ICD-10)[5]. According to statistics, in addition to neurological symptoms, patients with CP also have other symptoms, such as: pain (75%), intellectual disability (50%), inability to walk (33%), inability to talk (25%), epilepsy (25%), incontinence (25%), and blindness (10%)[4]. More than 100,000 Americans less than 18 years of age are suffering neurologic dysfunctions due to CP[6], while the life-span total economic loss from all new CP cases amounted to US$ 2–4 billion in China in 2003[7], placing a heavy financial burden on both developed and developing countries around the world. CP has become a major neurological disease that is harmful to children's health[8].

Stem cells have been used in many experimental CP animal models and achieved good results, depending on two essential

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Submitted: March 31, 2018. Revised: September 22, 2018. Accepted: September 24, 2018.

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properties: self-renewal (the capacity to generate identical cells) and multipotency (the capacity to generate many cell types).

Embryonic stem cells (ESCs) are considered the gold standard for pluripotency as they are able to differentiate into any cell type. However, three points limit their application: the possibility of undifferentiated ESCs leading to teratoma formation; the main source of ESCs, stillbirth, requiring ethical supervision; and the difficulty of creating a completely homogenous ESC culture[9]. Like ESCs, clinical application of induced pluripotent stem cells (iPSCs) has also been limited due to oncogenes that generate tumors[10].

It has been demonstrated that mesenchymal stem cells (MSCs) can also undergo adipogenesis, chondrogenesis, and osteogenesis upon induction, and secrete biologically active molecules. MSCs were first identified in bone marrow (BM)[11] and subsequently isolated from human and animal tissues such as umbilical cord blood (UCB), umbilical cord matrix (Wharton's jelly), adipose tissue, dental pulp, peripheral blood, and synovial fluid[12–15]. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimum criteria to define human MSCs: First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79alpha, CD19, and HLA-DR surface molecules. Third, MSCs must differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*[16]. However, the sampling process is invasive and affected by donor-derived tissue from BM. Moreover, MSCs easily age and have a limited lifespan[17].

Like BM, human UCB (hUCB) is a complex internal environment rich in a variety of stem/progenitor cell populations[18], such as hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), UCB monocytes (including T regulatory cells (Tregs) and monocyte-derived suppressor cells (MDSCs)) and MSCs[19,20]. The cellular fraction of the UCB has been referred to as a mononuclear cell (MNC) fraction that was collected from red blood cells and plasma[21].

When UCB cells are transplanted into the body, they are thought to play several roles: regenerative or regeneration support; tropic and nutritional functions in "homing"; or acting in a paracrine manner and regulating inflammatory effects and protection[22]. Numerous studies have scrutinized the neuroregenerative and neuroprotective potential of UCBs for treating CP, perinatal hypoxic-ischemic encephalopathy (HIE), periventricular leukomalacia, and adult stroke[23–25]. Here, we review the recent literature the current mechanisms underlying UCB-related stem cell types or components during CP-related treatment in order to evaluate the evidence for directing future studies on CP and treatment decision-making.

Discussion

UCB Composition Selection and Treatment Mechanism

The UCB components involved in cell therapy research are shown in Table 1. Compared with other types of stem cells, those from the UCB have advantages in terms of CP treatment safety and efficacy[22,25,34], ethics[35], non-neoplastic proliferation, accessibility, ease of preservation[36], and regulation of immune responses[25], based on findings in animal models and clinical trials[28,37–40]. In animal studies, UCB cell therapy has made substantial progress (migration to sites[41], improvement of functional loss[42], and prevention of white matter damage[43]). Encouragingly, in clinical trials, UCB therapy has been proven to be a safe, feasible, and potentially effective treatment for CP, particularly in motor function recovery in patients[44] and in post-acute phase neonatal brain injury[22].

The treatment mechanism can be summarized as follows:

Enhancement of Neuroregeneration by Homing. The stem cell niche is a special microenvironment that is suitable for stem cells. It mainly includes adjacent cells, extracellular matrix, and various cytokines. Stem cells achieve physiological steady-state growth, renewal, and differentiation through different signaling pathways including stromal-derived factor-1 (SDF-1)/ chemokine (C-X-C motif) (CXCR4), monocyte chemoattractant protein (MCP)-3/ cinnamoyl-CoA reductase (CCR), and hepatocyte growth factor (HGF) /c-met[45–47]. Chemotaxis refers to the concentration gradient of the ligands formed around the injured site by the release of inflammatory factors and chemokines surrounding the blood-activated stem cells. The homing effect of stem cells is the entire process of directed migration of the activated stem cells after injection into the target brain tissue along the concentration gradient, and performing regenerative functions[48].

The homing process begins with shear-resistant adhesive interactions flowing between cells and the vascular endothelium at the target tissue. Homing receptors expressed both on flowing cells (stem cells in blood) and related endothelial co-receptors cause cell-tethering and rolling contacts, thus mediating the interactions. Activation of integrins (including integrin β1) which play important roles in cell adhesion, migration, and chemotaxis set the anchorage for engrafted cells to make a hard adhesion. Finally, fixed "flowing cells" achieve their function through extravasation[49].

MSCs express the most common immune cell homing chemokine receptors including CXCR4, and the expression of SDF-1 after hypoxia-ischemia is significantly increased in the injured cerebral hemisphere and is mainly associated with astrocytes and glial cells[50–52]. Transplanted hUCB cells expressing the SDF-1 receptor CXCR4 migrate to the site of injury within 24 hours after induction of injury, further suggesting that SDF-1 is a potential chemokine for hUCB cell migration[53].

Immunohistochemical methods and small animal imaging systems have been used to track the distribution of hMSCs in the large blood vessels of brain injury after homing. The distribution of MSCs can be detected after transplantation of green fluorescent protein (GFP)-containing cells into the rat ventricles. Ten days after transplantation,

Table 1. The UCB Components Involved in the Research of Cell Therapy.

Cells	Proliferative potential	Related specific molecules	Differentiation potential
CB-MSCs[26]	>10 generations	Positive for CD13, CD29, CD44, CD49e, CD54, CD73, CD90, CD10, CD166 and HLA-ABC Negative for CD14, CD31, CD34, CD45, CD49d, CD80, CD86, CD106, HLA-DR	osteoblasts, chondrocytes, adipocytes, myoblasts and nerve cells
CB-USSCs[27]	Multiply population >40	Positive for CD13, CD29, CD44, CD49e, CD90, CD105, vimentin, CK8, CK18, CD10 and FLK1 Negative for CD14, CD33, CD34, CD45, CD49b, CD49c, CD49d, CD49f, CD50, CD62E, CD62 L, CD62P, CD106, CD117, glycophorin A and HLA-DR	osteoblasts, chondrocytes, adipocytes, cardiomyocytes, purkinje cells, liver and nerve cells
CB-CBEs[28]	168 times, The second generation	Positive for: CD34, CD133, CD164, SSEA-3, SSEA-4, Tral-60 and Tra1-81, Oct-4 Negative for: CD2, CD3, CD7, CD16, CD33, CD38 CD45, CD56, SSEA-1	bone, fat, skeletal muscle, blood vessels, liver, pancreas and nerve cells
CB-MPCs[29]	>28 times in 12 weeks	Positive for: CD14, CD31, CD44, CD45 and CD54 Negative for: CD49a, CD62E, CD73, CD90 and CD104	osteogenesis, endothelium, liver and nerve cells
CB-EPCs[30]	–	Positive for: CD14, CD31, CD34, CD133, VEGFR-2, Tie-1/2, VE-cadherin	blood brain barrier
CB-Tregs[21]	–	Positive for: CD14, CD80, CD83, CD86, HLA-DR, CD11b(MAC-1), Gr-1	–
CB-MDSCs[31]	–	Positive for: CD11b(MAC-1), Gr-1	–
Umbilical vein MSCs[32]	>20 generations	Positive for: CD13, CD29, CD44, CD49e, CD54, CD73, CD90, CD105, CD166 and HLA-ABC Negative for: CD14, CD31, CD34, CD45, CD49d, CD51/61, CD106, CD133, Cadherin-5, glycophorin A, HLA-DR and KDR	
WJ-MSCs[33]	Multiply population>80	Positive for: CD10, CD13, CD29, CD44, CD51, CD73, CD90, CD105 Negative for: CD14, CD31, CD33, CD34, CD38, CD40, CD40 L, CD45, CD56, CD80, CD86, CD117, HLA-DR	osteoblasts, adipocytes, chondrocytes cardiomyocytes and nerve cells

CB-MSCs: umbilical cord blood-derived mesenchymal stem cells; CB-USSCs: umbilical cord blood-derived unrestricted somatic stem cells; CB-CBEs: umbilical cord-derived embryonic-like stem cells; CB-MPCs: umbilical cord blood-derived multipotent progenitor cells; CB-EPCs: umbilical cord blood-derived endothelial progenitor cells; CB-Tregs: umbilical cord blood-derived T regulatory cells; CB-MDSCs: umbilical vein MSCs; WJ-MSCs: Wharton's jelly-derived mesenchymal stem cells; SSEA: stage-specific embryonic antigen; HLA: human leukocyte antigen; VEGF: vascular endothelial growth factor; MAC-1: macrophage-1 antigen.

MSCs with positive GFP expression could be seen in large vessels, and vascular endothelial growth factors were also higher in the transplanted group than in the control group[54]. In addition, Rahimzadeh et al. reported that transplantation of autologous MSCs in arrangement by HSCs improved HSC engraftment[49] and resulted in the production of anti-inflammatory macrophages for increasing tissue repair[55].

The effect of upregulating CXCR4 contributes to stem cell homing and colonization to damaged tissue. Intravenous delivery of genetically modified MSCs expressing CXCR4 proved to be a potentially useful and non-invasive therapeutic strategy for post-infarction myocardial repair[56], and the influence of CXCR4 expression on migration, proliferation, differentiation, and paracrine effects of MSCs was examined *in vitro*[57,58]. Overexpression of CXCR4 leading to enhanced mobilization *in vivo* and implantation of MSCs in ischemic areas[57] may improve the homing ability and colonization capacity of umbilical cord blood stem cells for neurological regeneration.

Secretion of Trophic Factors. There is a general consensus that after homing, stem cells secrete neurotrophic factors, cytokines, immunomodulatory factors, and angiogenic factors in a paracrine manner; these are proposed to be the most important therapeutic mechanisms. These factors influence target cells to modulate inflammation/apoptosis, activating progenitor cell proliferation and tissue repair to provide a good environment for cell survival, which is more direct and rapid after transplantation. The paracrine activity of stem cells is generally considered to comprise two main pathways: soluble factors and extracellular vesicles (EVs) (including exosomes).

Angulski et al. profiled the protein content of $CD133^{+}$-EVs isolated from UCB compared with BM-derived hMSCs

for better understanding of the functions in each vesicle type and delineating the appropriate use of each EV in therapeutic procedures[59]. For protein content, expanded CD133+-EVs might be better inducers or modulators of angiogenesis (e.g. von Willebrand factor) than hMSC-EVs, while hMSC-EVs might more efficiently induce/modulate differentiation (e.g. signal transducer and activator of transcription (STAT1)), phagocytosis, and innate immune responses (e.g. lactotransferrin (LTF) and complement component 1 binding protein (C1QBP)) of the target cells. However, typical actors of T-cell response inhibition or promoters of regulatory T-cell response in hMSC-EVs could not be found. Depending on the purpose of treatment, cord blood may be favored, since it is a more convenient extraction mixture containing MNC-EVs and MSC-EVs.

Neurotrophic Factors and Cytokines through EVs and in Soluble Molecules. The different types of EVs may implement regenerative functions directly through vesicle protein, miRNAs, or even trophic factors[60]. MSCs secrete EVs, which include exosomes and microvesicles, derived from the endosomal compartment, an isomeric mixture of microparticles (endoplasts), or directly from cytoplasmic membrane, resembling a saccular organelle-like structure. MSC-EVs have also been confirmed to bring about similar biological effects to cure various preclinical disease (including kidney, heart, and even brain injury models or patients[61,62]). Ophelders et al. investigated the protective effects and axonal growth of MSC-EVs in a preclinical model of preterm HIE brain injury in ovine fetuses by intravenous administration *in utero*. The results suggested that administration of EVs, rather than intact MSCs, is sufficient to exert effects and avoids potential concerns because the EVs lack their own metabolism and are hardly influenced by the environment *in vivo*[63]. Zhang et al. reported that native MSC-exosomes promoted axonal growth, and their designed tailored MSCs-exosomes could further boost this effect. Their designed tailored MSC-exosomes carried elevated miRNA-17-92 clusters relative to native MSC-exosomes, providing a potential therapeutic strategy to enhance axonal growth[26].

Soluble molecules are secreted from stem cells after fusion of secretory granules through the plasma membrane. Their effects are mediated via membrane receptor interaction with recipient cells[60]. Among them, granulocyte colony-stimulating factor (GCSF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) may be suitable for adjuvant stem cell therapy. GCSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) are hematopoietic hormones that promote the proliferation and differentiation of neutrophils[64]. The drug analogs of GCSF, filgrastim and lenograstim, are mainly used for the treatment of neutropenia and allogeneic or autologous BM transplant hypoplasia after restorative treatment[65]. These hematopoietic growth factors and their receptors were discovered 30 years ago and are highly expressed in neurons following brain injury. Other reports have suggested that GCSF may be a candidate for stem cell transplantation as a novel neurotrophic factor[66,67]. There is evidence that GCSF activates proteins of the Stat family and the PI3-K/Akt pathway to protect neurons from the intracellular pathways of hematopoietic cells[68]. In a randomized, double-blind, crossover study in South Korea, researchers evaluated the potential of nerve regeneration in intraventricular GCSF after infusion of mobilized peripheral blood mononuclear cells (mPBMCs) in a clinical trial for CP (trial registration: ClinicalTrials.gov, NCT02983708). Apart from demonstrating the potential for neural regeneration of intravenous GCSF followed by mPBMC reinfusion, they also concluded that the observed improvement in neurodevelopment may be due to GCSF alone, rather than the effect from reperfused mPBMCs. Future studies should examine the benefit of reinfusion of mPBMCs alone at higher concentrations without GCSF[69]. In the JAK / Stat pathways, the erythropoietin (EPO) gene shows a highly similar expression profile between neural and hematopoietic stem cells. EPO receptors are cytokine-type transmembrane proteins. EPO receptors that have neuroprotective effects are heterodimers and constitute common members of the receptors for cytokines IL5, IL3, and GM-CSF[70]. In the PI3K-Akt signaling pathway, Akt-mediated activation of anti-apoptotic neurons is crucial. GCSF activates the PI3K-Akt signaling pathway, and EPO is a major contributor to neuroprotection via this pathway. GCSF and EPO are also involved in ERK1/2 kinase pathways, and both their receptors lack intrinsic tyrosine kinase activity[71–73].

The roles of BDNF and GDNF in regulating neuronal development, regeneration, and survival have been repeatedly confirmed. It has now been found that adding BDNF can differentiate over-expressed hUCB-MSCs into an increased number of neuron-like cells, and can upregulate neuronal phenotypic marker expression.

Although the formation of functional nerve cells and the establishment of axonal connections are among the goals of cell therapy for CP, administration of neurotrophic factors alone may support the function of existing cells and build a good growth environment for newborn nerve cells and axons.

Angiogenic Factors through EVs and in Soluble Molecules. Mononuclear cell-derived ($CD133^+$ cell) EVs have been tested in various disease models, including neurological diseases.[74,75] Expanded $CD133^+$ or endothelial-like cells (EPC)-EVs containing vascular endothelial growth factor (VEGF) and angiogenin appear to act primarily through stimulation and modulation of angiogenesis[76]. VEGF served as an ideal candidate additive, but its subtypes differ greatly in generating blood vessels. Beerens et al. reported that, unlike mouse ESCs or iPSCs, culturing multipotent adult progenitor cells with VEGF-A produced a mixture of arterial, venous, and lymphatic endothelial cells. Addition of

VEGF-C did not have this effect[77]. Angiogenin may be a key factor, since angiogenin signaling attenuated the positive effects of the EPC secretome. *In vivo*, treatment with the EPC secretome increased vascular density, myelin, and mature oligodendrocytes in white matter, and ameliorated cognitive function in a mouse model of hypoperfusion[78].

Immunoregulation and Neuroprotection. UCB mononuclear/whole blood cells in the perinatal ischemic and hypoxic brain model can reduce the inflammatory response to treat injury[21]. To evaluate whether transplanted cells relieve neuroinflammation, there are two indicators: (1) reduce the infiltration of $CD4^+$ T cells into the brain; and (2) reduce microglial activation. McDonald and colleagues confirmed that all UCB cell types except EPCs have CNS immunoregulatory capacity. Tregs and monocytes are present in the normal body at a considerable level, and are indispensable in the regulation of peripheral and central immune responses[21].

Some specific subsets of Tregs suppress T-cell proliferation. After transplantation, IL-4-produced Th2 cells and Tregs occurred frequently both within the brain and in peripheral tissues. In general, it is beneficial to coordinate an immunomodulatory macrophage response, reduce microglial activation, and to protect against tissue injury[79]. However, McDonald et al. only found a significant increase in the proportion of IL-4-producing Th2 cells after UCB treatment alone, without an increase in the number of Tregs[21]. Whether the uniqueness of this upregulation of inflammatory factors can serve as an entry point for repairing neural tissue from the peripheral pathway remains to be explored. MDSCs have the potential to be used as novel seed cells due to their comprehensive anti-inflammatory effects[80,81] (modulating innate and adaptive immune responses by promoting T lymphocyte apoptosis). However, it is striking that monocytes did not reduce microglial activation but instead activated the neuroinflammatory response and assisted in gathering T lymphocytes in the brain. McDonald et al. proposed that MDSCs may not be the only $CD14^+$ monocyte in the trial, and future experiments need to study more specific surface markers for MDSCs.

In addition, the aforementioned secretory cytokines and immune factors also play a role in immune regulation. MCP-1, interleukin (IL)-6, IL-8, and IL-10 secretion were also observed in UCB. MCP-1 in astrocytes has been reported to be critical for inflammation development. After UCB treatment for 48 h in both hippocampal and striatal ischemic tissues, the ischemic extracts demonstrated that growth-regulated oncogene/CINC-1 (GRO/CINC-1, the rat equivalent of human IL-8) and MCP-1 were expressed in a time-dependent pattern. TNF-α-induced astrocytes have been associated with a variety of pathological situations. MCP-1 regulates TNF-α through the activated protein kinase signaling (AMPK) pathway[81].

HSCs have long been considered to have neuroprotective potential by stimulating and participating in angiogenesis[82-84], and hMSC-EVs containing immunomodulatory factors were proved to act in several processes in the modulation of the immune response (developmental, maturation, and induction process)[85]. MSC-EV administration alone not only improves brain function but also avoids the development of intracerebral inflammation[63]. Studies have shown that MSC-EVs can improve inflammation-induced neuronal degeneration, reduce microglial proliferation, and prevent reactive astrocyte proliferation. Short-term myelin deficiency and long-term microstructural abnormalities in white matter can be improved by administration of MSC-EVs, and is an active research direction[86]. Different cells release different inflammatory chemokines that mediate the innate and adaptive immune systems. Currently, 50 chemokines and 20 different chemokine receptors have been discovered[87].

It must be pointed out that the three mechanisms of action are inextricably linked during the course of cell therapy. The homing cells colonize the injured site and participate in the formation of neurons, glial cells, axons, and blood vessels surrounding tissues. The homing cells release neurotrophic factors in a paracrine manner to promote the regeneration of neurons and blood vessels and release inflammatory and anti-inflammatory factors to protect the growth environment. Early inflammation is beneficial, and Treg regulation is essential for regeneration. The majority of non-homing cells also release large amounts of soluble molecules or transmit angiogenic factors, cytokines, or even miRNAs in the form of EV-cells to promote regeneration. More tissue regeneration magnifies the effect on repair damage (Fig. 1). In order to elaborate on the role of each cell or factor in UCB and to find suitable complementary treatment options, we describe the three mechanisms in isolation.

Outcomes and Outlook

Clinical Trials

A total of 18 clinical trials for CP treatment using UCB have been registered from clinicaltrials.gov since June 25, 2018 (Table 2) (enrollment data not uploaded in NCT01486732 and NCT03203941), including 10 completed studies (with the exclusion of HIE). However, when McDonald et al. published their review (September 2017), there were only 11 clinical trials for CP (only four completed). More clinical trials are now being designed and completed, indicating that UCB is a valid choice for CP.

Nevertheless, the conclusions of ongoing and completed trials are positive but still limited. One of the reasons is that most of the trials are still open-label, single-group studies. This phenomenon reflects the urgent feelings of parents and research designers themselves, who want to obtain curative effects in a short time. Because of the long course of treatment, it may be difficult for some of the children in the control groups to have sufficient time and funds to proceed. Another reason is that there are too many variables to control. Both McDonald et al.[25] and Novak et al.[22] mention in their papers that the sample size was small. However, we

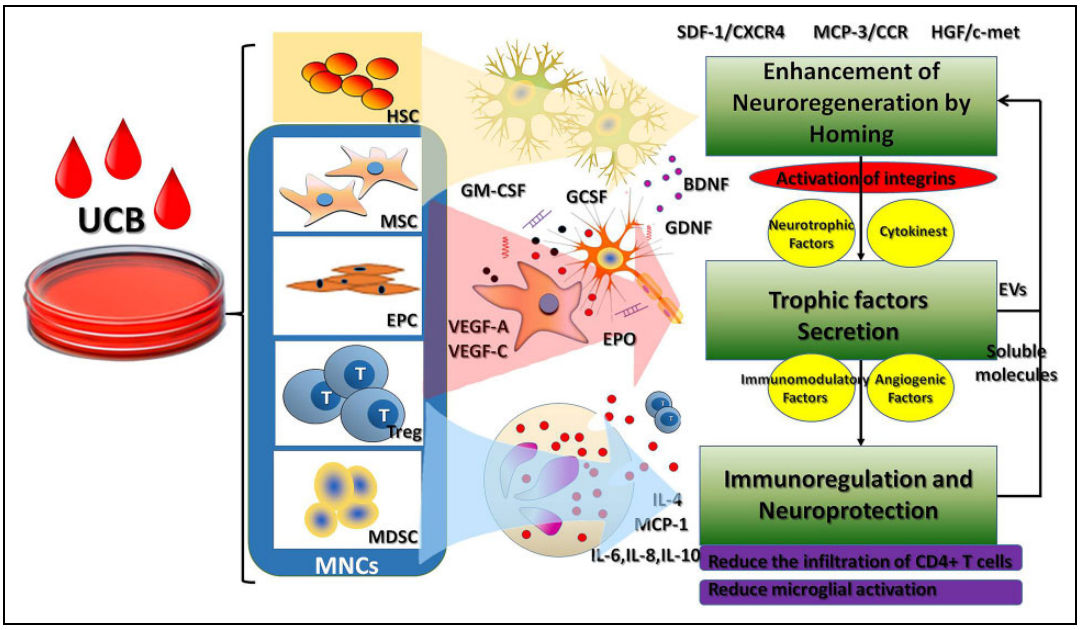

Figure 1. Therapeutic effects and related mechanisms of major cellular components of cord blood on cerebral palsy. Five common components were isolated from collected cord blood (HSPs, MSCs, EPCs, Tregs, MDSCs) and their therapeutic effects are achieved, respectively, through the mechanisms homing and neuroregeneration, trophic factor secretion, and immunoregulation and neuroprotection. The three treatment mechanisms are also interrelated.

believe that the reason for this "small samples impression" is that the common conditions of the samples are too few to be informative (too many variables designed in the study).

Treatment Effects and Adverse Reactions

In Rizk et al.'s article[34], 12 of the UCB therapy studies are for patients with CP. The results in nine studies were considered to be probably effective (patients 201/276) and four of these studies have control groups. Sixty-seven patients had adverse reactions: fever (20/67), nausea and vomiting (13/67), rash (4/67), seizure (3/67), and others (4/67). With regard to cell types, 31 studies are for mononuclear cells, 20 studies for MSCs[26], and 11 for autologous cells.

NCT01193660, randomized and parallel assignment, enrolled 105 participants from age 10 months to 10 years for a 6-month observation period. Some 31/35 CP patients joined a cell therapy group (total nucleated UCB cells > 3×10^7/kg intravenously and erythropoietin, twice a week for 4 weeks with the dosage of 500 IU/kg twice intravenously and 250 IU/kg six times subcutaneously, and active rehabilitation). 33/36 and 32/34 patients were divided into erythropoietin and rehabilitation group and rehabilitation-only group. The study demonstrated that cell therapy improved children's functions in most outcome measures (motor performance, gross motor function, cognitive neurodevelopment outcome, motor neurodevelopment outcome; comparison of changes in brain glucose metabolism using by brain 18F-FDG PET, functional independence in daily activities, muscle strength, and hand function). These were no differences in serious adverse events (pneumonia, seizures, influenza, urinary tract infection, death) rates (3/35 in UCB with erythropoietin and rehabilitation group, 3/36 in erythropoietin and rehabilitation group, 3/34 in rehabilitation-only group)[88]. NCT01147653, a double-blind, placebo-controlled, crossover study of a single intravenous infusion of $1–5\times10^7$ total nucleated cells per kilogram of autologous cord blood (ACB) for children with CP aged 1–6 years enrolled 63 participants. In an analysis 1 year post-ACB treatment, those who received doses $\geq2\times10^7$/kg demonstrated that appropriately dosed ACB infusion improves brain connectivity and gross motor function (significantly increases in Gross Motor Function Measure-66 (GMFM-66) scores, Peabody Developmental Motor Scales-2 Gross Motor Quotient scores and normalized brain connectivity)[89]. It is noteworthy that the number of gastrointestinal disorders (2/63, 3.17%) in the trial was much less than that in NCT01193660 (27/35, 77.14%), but the number of children with respiratory-related infections (9/63, 14.28%) was far higher than the control group (2/63, 3.17%).

Collectively, these results lead to several conclusions. UCB is one of the most used cell types in clinical trials for CP with reliable safety and efficacy. Some 72.8% of patients with CP benefit from UCB-related cell therapy[25]. However, with the exception of NCT03473301 being recruited, no independent controlled trial results have been published for each component in UCB so far. The general consensus of administration time is "the sooner the better"[90]. Before the UCB test, a dose safety test must be performed[22,23]. In a preclinical animal study, after the first 24 hours of initial

Table 2. Clinical Trials Using Umbilical Cord Blood for the Treatment of Cerebral Palsy. Information Obtained from *ClinicalTrials.gov*.

NCT Number	Title	Status	Interventions	Study type	Outcome measure	Population	Research purposes
01639404	Umbilical Cord Blood Therapy for Children With Cerebral Palsy	Complete	UCB+ Rehabilitation	Single Group; Open Label	Motor Performance; Standardized Gross Motor Function; Cognitive; Visual Perception Test;	Enrollment:17 (6M-12Y)	Neuroregeneration; Trophic secretion;
01528436	Umbilical Cord Blood Therapy for Cerebral Palsy	Complete	UCB+ Rehabilitation	Randomized; Parallel; Quadruple	Gross Motor Function; Cognitive; Visual Perception Test; Muscle Strength; Brain Glucose Metabolism;	Enrollment:37 (6M-20Y)	Neuroregeneration; Trophic secretion;
01193660	Allogenic Umbilical Cord Blood and Erythropoietin Combination Therapy for Cerebral Palsy	Complete	UCB+EPO+R/UCB/ EPO	Randomized; Parallel; Quadruple	Gross Motor Function; Cognitive; MRI; Brain Glucose Metabolism; 18F-FDG; Muscle Strength	Enrollment:105 (10M-10Y)	Neuroregeneration; Trophic secretion;
02599207	Assessment of the Safety of Allogeneic Umbilical Cord Blood Infusions in Children With Cerebral Palsy	Active, not recruiting	Sibling UCB	Non-Randomized; Open Label	adverse events; gross motor function	Enrollment:15 (1Y-6Y)	Neuroregeneration; Trophic secretion; Neuroprotection
02025972	Allogeneic Umbilical Cord Blood Therapy in Children With CP	Complete	Allogeneic UCB	Single Group Assignment; Open Label;	Cytokine analysis; Gross Motor Function; Cognitive;	Enrollment:10 (up to 15Y)	Neuroregeneration; Trophic secretion; Neuroprotection
01072370	Safety and Effectiveness of Cord Blood Stem Cell Infusion for the Treatment of Cerebral Palsy in Children	Recruiting	Autologous UCB	Randomized; Crossover Assignment; Blind	Safety, follow-up over one year with clinical and laboratory evaluations; efficacy, Gross Motor Function	Enrollment:40 (1Y-12Y)	Neuroregeneration; Trophic secretion;
01988584	Safety and Effectiveness of Banked Cord Blood or Bone Morrow Stem Cells in Children With Cerebral Palsy (CP)	Complete	Autologous SC/ Saline	Randomized; Crossover Assignment; Triple	Safety Late functional outcome	Enrollment:20 (2Y-10Y)	Neuroregeneration; Trophic secretion; Neuroprotection
03473301	A Study of UCB and MSCs in Children With CP: ACCeNT-CP	Not yet recruiting	Allogeneic UCB; MSC	Randomized; Parallel; Single	GMFM-66 score Adverse Event	Enrollment:90 (24M-60 M)	Neuroregeneration; Trophic secretion;
01147653	A Randomized Study of Autologous Umbilical Cord Blood Reinfusion in Children With Cerebral Palsy	Complete	Autologous UCB; Placebo	Randomized; Crossover Assignment; Quadruple	(GMFM-66) Score; Peabody Gross Motor Quotient; CP-QOL Score; MRI;	Enrollment:63 (12M-6Y)	Neuroregeneration; Trophic secretion;
02866331	GCSF and Autologous Cord Blood Infusion in Cerebral Palsy	Recruiting	GCSF; CB; Placebo	Randomized; Parallel; Quadruple;	Safety; efficacy;	Enrollment:88 (2Y-10Y)	Neuroregeneration; Trophic secretion; Neuroprotection
03130816	Mechanism of Allogeneic UCB Therapy in Cerebral Palsy	Recruiting	Allogeneic CB	Single; Open Label	GMFM; mRNA assay; GMPM	Enrollment:90 (10M-20Y)	Neuroregeneration; Trophic secretion; Neuroprotection
03087110	Stem Cells in Umbilical Blood Infusion for CP	Active, not recruiting	sibling donor CB	Single; Open Label;	gross motor function; cognitive;	Enrollment:12 (1Y-16Y)	Neuroregeneration; Trophic secretion;
01991145	Allogeneic UCB Therapy With EPO in Children With CP	Complete	UCB+R/EPO	Randomized; Parallel; Quadruple;	Gross Motor Function; Cognitive;	Enrollment:92 (10M-6Y)	Neuroregeneration; Trophic secretion;

(continued)

Table 2. (continued)

NCT Number	Title	Status	Interventions	Study type	Outcome measure	Population	Research purposes
02236065	Combination Therapy of Cord Blood and GCSF for Patients With Brain Injury or Neurodegenerative Disorders	Complete	UCB+ GCSF	Single; Open Label;	Berg Balance Scale; Gross Motor Function; ALSFRS-R; UPDRS	Enrollment:10 (19Y-75Y)	Neuroregeneration; Trophic secretion;
01506258	Autologous Stem Cells in Newborns With Oxygen Deprivation	Unknown status	SC+ Observation	Non-Randomized; Parallel; Open Label; Prevention	Effects;	Enrollment:20 (37W-42 W)	Neuroregeneration; Trophic secretion;
01929434	Efficacy of Stem Cell Transplantation Compared to Rehabilitation Treatment of Patients With Cerebral Paralysis	Complete	SC+ rehabilitation	Randomized; Parallel; Single	Gross Motor Function; Routine Blood Test	Enrollment:300 (1Y-14Y)	Neuroregeneration; Trophic secretion;
01147653	A Randomized Study of autologous Umbilical Cord Blood Reinfusion in children with cerebral palsy	Complete	Autologous UCBs	Randomized, Double-blind; Crossover Assignment	Improvement of standardized measures of neurodevelopmental function at 2 years	Enrollment:63 (12M-6Y)	Neuroregeneration; Trophic secretion;

insult, UCB mononuclear cell therapy as an early intervention showed a greater benefit in an HIE model[23]. Early UCB intervention mainly exerted immunoregulatory and neuroprotective effects. Mononuclear cells seem to play a major role. The mechanism of neuroregeneration produces effects long (6 months or longer) after injury. Common adverse reactions are fever, rash, etc. At present, the efficacy and safety of autologous UCB are superior to that of allogeneic UCB. The different medicines and cell types have shown beneficial effects in clinical studies, but combination strategies may be the future of neural regeneration[91].

Potential Cell Sources and Dose

In the current 18 trials, there are six types of tests using different sources (UCB+ Rehabilitation; UCB+EPO; Autologous UCB; Allogeneic (sibling or otherwise) UCB; GCSF) and three different doses (more than 10 M/kg body weight; more than 30 M/kg body weight; not mentioned). It is generally believed that 10 M/kg body weight is a safe dose for intravenous administration. Intravenous administration of autologous umbilical cord blood cells (UCBCs) therapy may be the safest and most feasible because UCB has been used for hematopoietic stem cell transplantation for decades[92]. Allogeneic cells for CP research and trials are readily available, primarily from privately and publicly banked UCB units, and the treatment of non-invasive injuries is easily accepted.

The blood cell collection, separation, and storage of UCBCs should be regulated to prevent infection[93]. Jantzie et al. suggested that although UCB-MNCs and stem cells are relatively easy to obtain, the number obtained from each infant in each batch is variable and heterogenous, rendering the assessment of efficacy inconsistent[94]. Therefore, the exact mechanism and optimum content of each component of UCB still need to be explored.

Candidate Situations

There have been numerous trials involving various conditions and recruitment of both adults and children. The degree of cooperation between the candidates under 6 years of age for evaluation and treatment is very low. In terms of efficacy, the treatment results for younger children seem to be more robust. To date, only six trials had treatment groups of 0–6 years old. It may be beneficial for national research teams to demonstrate cooperation, share results, and standardize testing standards.

Jantzie et al. claim that preterm babies may not be the best candidates for autologous stem cell transplants, as the collection volumes are proportional to gestational age[94]. McDonald et al. proposed that each individual UCB unit has different proportions and changes throughout gestation[27], meaning that preterm UCB has different cell contents compared with that of term UCB[95].

Optimal Administration Route

Currently reported routes of administration are intraventricular, intrathecal, intranasal, intramuscular, intra-arterial, or intravenous[96–98]. Intraventricular and intrathecal injections are theoretically straightforward but invasive, in which the risks are unacceptable to children. Common complications include meningeal irritation such as nausea and vomiting, intraventricular hemorrhage, and subarachnoid hemorrhage.

The intravenous route is the most commonly used non-invasive protocol. Tracer methods show that a significant number of cells circulating through the system are colonized in the lungs[99,100]. The most serious adverse effect of intravenous and arterial administration is pulmonary embolism, which is related to fast administration or excessive dose. Systemic pathways have the potential to modulate inflammatory responses, but a significant portion remains in other organs, and many do not cross the blood–brain barrier[97]. Both Jantzie et al. and Kiasatdolatabadi et al. considered that local administration may be more feasible, and injection location even within the brain may be important[94,101].

Conclusions

We have reviewed the current mechanism of UCB-related stem cell types or components in CP-related therapy. UCB is beneficial for clinical use and its underlying mechanism has been studied, and meaningful progress made in both preclinical settings and clinical trials. However, several pressing issues for bringing stem cells into practice exist: (1) identifying the source of low-cost stem cells with high purity; (2) selecting the stem cell type with the best efficacy and safety; (3) in-depth study of the treatment mechanism of stem cells; (4) identifying administration route and dose; and (5) unifying effective evaluation criteria and follow-up work. Our review summarizes the features of active components in UCB and the therapeutic mechanism of action for treatment suggestions, but there needs to be much more research before its safe clinical use.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (No.81271412, 81471308, 31500793), International S&T Cooperation Project of the Ministry of S&T of China (No.2010DFR30850), the Scientific Research Foundation for Returned Overseas Chinese Scholars, and the Doctoral Scientific Research Foundation of Liaoning Province (No. 201501158).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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