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COULD AUTOLOGOUS CORD BLOOD STEM CELL TRANSPLANTATION TREAT CEREBRAL PALSY?

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Abstract

The young human brain is highly plastic and thus early brain lesions can lead to aberrant development of connectivity and mapping of functions. This is why initially in cerebral palsy only subtle changes in spontaneous movements are seen after the time of lesion, followed by a progressive evolution of a movement disorder over many months and years. Thus we propose that interventions to treat cerebral palsy should be initiated as soon as possible in order to restore the nervous system to the correct developmental trajectory. One such treatment might be autologous stem cell transplantation either intracerebrally or intravenously. All babies come with an accessible supply of stem cells, the umbilical cord, which can supply cells that could theoretically replace missing neural cell types, or act indirectly by supplying trophic support or modulating inflammatory responses to hypoxia/ischaemia. However, for such radical treatment to be proposed, it is necessary to be able to detect and accurately predict the outcomes of brain injury from a very early age. This article reviews our current understanding of perinatal injuries that lead to cerebral palsy, how well modern imaging might predict outcomes, what stem cells are yielded from umbilical cord blood and experimental models of brain repair using stem cells.

Keywords

• Cerebral palsy • Embryonic stem cell • Haematopoietic stem cell • Hemiplegia • Mesenchymal stem cell • Periventricular white matter injury • Umbilical cord blood

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1. Introduction

In the developed world the incidence of cerebral palsy is high, around 2 per 1000 live births or higher [1,2]. It is therefore a common condition that causes disability throughout life, which is often severe. Cerebral palsy is an umbrella term that covers a number of conditions including cerebellar ataxia and basal ganglia disorders amongst their symptoms, but this article will concentrate on the most common condition, spastic cerebral palsy (80% of cases) which arises out of insults primarily to the cerebral cortex and associated, sub-cortical white matter [3]. Causal pathways to cerebral palsy are many and may interact with each other, indeed multiple causes, including a genetic predisposition to infarction, may need to interact to produce a clinically significant injury [3,4].The most commonly encountered causes are summarised in Figure 1 and include periventricular white matter injury in premature babies, which results from hypoxia/ ischaemia (H/I) in the periventricular regions around the lateral ventricles. This results primarily in damage to the subplate and developing sub-cortical axon tracts of the intermediate zone whilst the overlying grey matter is relatively spared. It generally results in spastic diplegia. In all, bilateral spasticity has a prevalence of 1.2/1000 live births [5]. Unilateral spasticity and weakness is also common (prevalence 0.6/1000 live births) with roughly one third of cases resulting from focal periventricular white matter lesions and one third involving cortical or deep grey matter lesions, mainly as a result of infarcts of the middle cerebral artery. A further fifth of such cases result from brain maldevelopments, mainly focal cortical dysplasia or unilateral schizencephaly [6]. More severe hypoxia or anoxia at the time of birth is associated with widespread injury of white and grey matter resulting in spastic quadraparesis along with severe cognitive deficits. In all cases perinatal lesions of the corticospinal system give rise to subtle but observable changes in spontaneous general movements without giving rise to the traditional neurological signs observed in older children and adults [7,8]. There is a progressive evolution of the movement disorder over months and years.

2. Perinatal brain injuries: causes and outcomes

2.1 Perinatal stroke leading to spastic hemiplegia

The incidence of stroke is highest in prematurely born babies compared to any other time of life, although the incidence for babies born at term is also high [9]. Approximately two thirds of children who suffer from perinatal stroke develop cerebral palsy and nine tenths of these will develop hemiplegic cerebral palsy [10]. The impact on individuals and their families is substantial [11] and on reaching adulthood, affected individuals frequently do not have sufficiently skilful bimanual dexterity to be fully

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independent, which has a significant life-long impact on their mental health, quality of life and earning capacity [12] despite the majority having cognitive capacities within the normal range [13].

The outcome after an adult onset stroke is largely determined by the extent of the initial brain injury and motor recovery occurs if a critical amount of corticospinal system function has been spared at the time of the lesion [14]. However, this is not the case for a perinatal stroke and infants with a significant corticospinal projection from the infarcted cortex soon after the stroke, detected by transcranial magnetic stimulation, can still have a poor motor outcome [15]. A longitudinal study has shown that in the first 24 months after stroke, progressive loss of corticospinal projections from the affected cortex may occur. The findings at 24 months were predictive of outcome; those in whom transcranial magnetic stimulation (TMS) fails to evoke responses in the affected limb have a poor outcome, failing to develop functional use of their paretic hand, whilst those in whom a response has been preserved have a better outcome, developing functionally useful dexterity in childhood [15].

After a unilateral stroke, although a corticospinal projection may be present, activity in the infarcted cortex is suppressed. Thus it has been proposed that surviving, but not very active, corticospinal projections may lose out in competition for spinal cord synaptic space leading to these projections being withdrawn as their potential targets are taken over by more active ipsilateral corticospinal projections from the unaffected hemisphere and also by proprioceptive muscle afferents [16-18]. Evidence from animal experiments supports this hypothesis [19-22]. Stem cell transplants that could provide trophic support to the infarcted motor cortex, or perhaps even replace lost corticospinal neurons, might help establish a more functionally active corticospinal projection from the infarcted side.

2.2 Periventricular white matter injury

Periventricular white matter injury (PVWMI) is commonly seen in premature and low birth

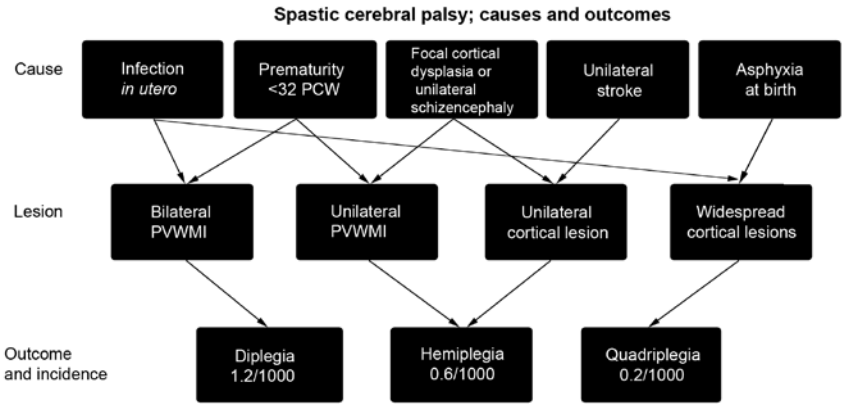

Figure 1. A summary of the causes of spastic cerebral palsy, and the particular outcomes they lead to. Asphyxia at birth may arise from prolapsed cord, intrapartum haemorrhage, uterine rupture or maternal cardiac arrest. As arrows indicate, multiple causes may combine to produce cerebral palsy [3] and may also interact with subtle genetic variations in individuals that cause predisposition to stroke [147]. PCW: post-conceptional weeks; PVWMI: periventricular white matter injury.

weight babies. It leads to lesions which consist of, at one end of the spectrum, regions of hypomyelination and at the other end, cystic lesions of the subcortical white matter adjacent to the external angles of the lateral ventricles [23] that largely leave the cortical grey matter intact [24]. Although neuroimaging studies in premature infants show some reduction of cerebral cortical gray matter volume and reduced gyrification [25,26] PVWMI is the most important cause of cerebral palsy in prematurity and its incidence, along with the severity of cerebral palsy, have actually increased over time as medical advances have led to a greater survival rate for premature infants [27]. Its etiology is multifactorial and possibly combinatorial, involving both prenatal and perinatal factors that may include genetic causes, ischaemic-reperfusion failure, growth factor deficiency, and infection or inflammation ante- or postnatally [27-29].

Age dependent regional susceptibility is a major characteristic of PVWMI. The highest susceptibility in the human brain is between 24 and 32 weeks gestation; a stage of vascular development that leaves the periventricular regions at risk of hypoperfusion and hypoxia [30]. At this stage of development the subcortical white matter is populated predominantly by premyelinating oligodendrocytes [31,32], including precursor cells and immature oligodendrocytes. Such cells are more vulnerable than mature oligodendrocytes to a variety of H/I injury-related insults including glutamate receptor-mediated excitotoxicity [33,34] and also arrested development [35,36] which may arise out of oxidative stress on the cells or inhibition of differentiation by extracellular components of any astrocytic scar [37]. Furthermore, the external angles of the lateral ventricles in particular are a location for accumulations of microglia cells at what is regarded as a "crossroads" site for various axonal projections. These microglia may be involved in axonal guidance but also provide a substrate for an enhanced inflammatory reaction in PVWMI [38] producing pro-inflammatory cytokines, as well as excitotoxic glutamate and free radicals [39,40]. In animal models, blocking microglial activation can protect the brain [41].

In addition to white matter injury, the transient subplate zone of the developing cortex peaks in size between 24 and 32 gestational weeks [42]. It is located between the periventricular white matter and the smaller, developing cortical plate and has been shown to be vulnerable to H/I injury in the preterm [43]. It is relatively more mature than the cortical plate, having a better developed synaptic circuitry [44] and a higher expression of glutamate receptors that make its neurons relatively more vulnerable to excitotoxic injury

[45,46]. Subplate neurons play an essential role in the development of connections between thalamus and cortex and of connections within the cortex [47,48]. The time period of vulnerability to PVWMI, with its secondary damage to axon tracts and to subplate neurons, coincides with the timing of thalamocortical and cortico-cortical [49] and corticospinal synaptogenesis [50] and thus can be viewed as perturbing the trajectory of sensorimotor development at a crucial stage leading to aberrant development of connectivity and mapping of functions [16].

2.3 Predicting the outcome of perinatal brain injury

At least 80% of babies born prior to 33 gestational weeks manifest at least modest lesions of the white matter when viewed by ultrasound imaging. However, many of these have resolved themselves before term equivalent age (TEA) such that less than 10% show moderate or severe white matter lesions when examined by magnetic resonance imaging (MRI) [51]. A previous study had shown that there is a significant correlation between severe or moderate white matter abnormalities observed by MRI at TEA and a diagnosis of cerebral palsy and other neurodevelopmental disorders at two years of age [52]. However, as has already been pointed out [53] only 31% of the cohort actually developed cerebral palsy and 51% a neurodevelopmental disability of any kind. Furthermore, although only 5% of the cohort developed cerebral palsy when presenting with no or mild white matter abnormalities at TEA, and these were not significant predictors of a poor outcome, the greater size of the cohort presenting in this way meant that still a large number of infants were affected. Clearly, we are not at the stage yet when we could recommend invasive procedures to correct only potentially adverse outcomes. However, advances in imaging, including functional MRI (fMRI) and diffusion tensor imaging in newborns to assess long range connectivity and cortical network activity [54,55] and in conjunction with electro-encephalographic investigations [56] may increase our powers of prediction.

3. Stem cells from umbilical cord blood

3.1 Potential for therapy

Human umbilical cord blood stem cells hold a tremendous potential for therapy. They have considerable practical and ethical advantages over other stem cells, as they are isolated from umbilical cord non-invasively without any side effects to either the baby or the mother, and have been used in clinical practice for around forty years in the treatment of blood diseases (lymphoblastic leukaemia at first, [57]). More recently their use has been extended to various regenerative medicine and tissue engineering approaches, including Type 1 Diabetes [58-61], myocardial repair and regeneration [62] and potential treatments for neurological conditions (see below).

Umbilical cord blood can even be contemplated as a source of stem cells for allogenic transplantation to older individuals with cerebral palsy as it is an abundant stem cell source. The global birth rate is over 200 million per year and umbilical cord blood can be stored and cryopreserved in cord blood banks for later uses [63]. Many cord blood banks have been established worldwide, with 43 public cord blood banks in 26 countries [64]. Due to the naive nature of a newborn's immune system, transplantation of cord blood results in fewer acute and chronic graft versus host disease incidences [65,66]. Transplantation of cord blood was shown to be associated with a lower risk of viral transmission than bone marrow transplantation [67].

Umbilical cord blood cells can be considered as an intermediate age stage between embryonic and adult stem cells, having higher proliferating potential and longer telomeres than other somatic stem cells [68,69]. Umbilical cord blood contains a unique, heterogeneous mixture of stem and progenitor cells in numbers not seen in any other locations, and includes embryonic-like stem cells, hematopoietic stem cells, endothelial stem cells, epithelial stem cells, mesenchymal stem cells (MSCs), unrestricted somatic stem cells and even neuronal-like stem cells [63,70-74].

3.2 Embryonic-like stem cells.

Embryonic-like stem cells, with certain embryonic stem cells characteristics, have been purified from the mononuclear fraction of cord blood using fluorescence activated cell sorting and immunomagnetic depletion strategies [71,72,75,76]. These immature stem cells are positive to pluripotency markers expressed in embryonic stem cells such as Oct-4, Sox-2 and Nanog [63,76,77]. Evidence has been provided for their successful differentiation into specialized cells from all three germ layers [63,78,79]. They carry minimal mutations making them a safe source of stem cells. Cord blood stem cells lack major drawbacks identified in other stem cell types. Embryonic stem cells (ESCs) lack a fully developed G1 check point, and therefore have a high chance of acquiring mutations and transformation into tumour cells [80,81]. Induced pluripotent stem cells (iPS) reprogrammed from adult stem cells with characteristics similar to embryonic stem cells, are still at risk of tumourigenesis and diminished cellular functions due to difficulties harvesting adult cells, inefficient reprogramming and the accumulation of genetic errors ([82,83].

3.3 Mesenchymal stem cells

A population of MSCs has also been purified from umbilical cord blood, with different developmental and morphological characteristics to umbilical cord blood pluripotent stem cells [80]. Cord blood MSCs show similar cellular, morphological and differentiation potentials as bone marrow-MSCs including a potential for neural differentiation [84]. One major limiting factor is the low frequency of MSCs in cord blood, which means that only a low cell dose is available for transplantation, another is the inconsistency of successful isolation of MSCs from umbilical cord blood. The total nucleated cell dose (TNC) and the hematopoietic colony-forming cell (CFC) transplanted per kilogram (kg) of body weight of the recipient correlates with outcomes [85-88]. For this reason, umbilical cord blood transplantation remains significantly more successful in younger children than for adolescents and adults with leukaemia and lymphoma [89]. However, Zhang *et al.* recently

reported 90% success in isolation of cord blood MSCs by density gradient purification, without need for immunoaffinity methods, from cord blood units having a volume less than 90 ml if collected within two hours after the donor's birth [90]. These cord blood-MSCs exhibited a higher proliferation rate and could be expanded to the order of the 1×10^9 cells, the number required for cell therapies. The cells showed karyotype stability and an immunomodulatory effect even after prolonged expansion [90].

3.4 Neurogenic potential of umbilical cord blood stem cells

During the last decade, numerous groups have claimed to demonstrate the generation of neural cells from cord blood progenitors with different experimental approaches, including supplementation with growth factors, such as nerve growth factor (NGF) [91], γ-interferon [92] and brain-derived neurotrophic factor (BDNF), as well as induction with chemical agents, such as retinoic acid [93,94] dimethylsulfoxide [95] and beta-mercaptoethanol [96]. These induced neuronal cells were characterized by the expression of typical neuronal markers specific to different stages of neural development [91,92,97,98] and by electrophysiological properties that show some similarity with primary neurons [99-101]. For instance, when the electrical activity of cord blood-derived CD34-/CD45- differentiating cells was recorded using whole-cell patch-clamp, functional voltage-dependent potassium and sodium channels and ligand-gated channels, such as acetylcholine, gamma-aminobutyric acid, glutamate, glycine, 5-hydroxytryptamine and dopamine receptors, were identified [100]. Most recently, ESC-like cells from human cord blood have been shown to recapitulate the differentiation pathway of glutamatergic neurons of the cerebral cortex by sequentially expressing the transcription factors PAX6, TBR2 and TBR1, followed by other neuronal markers including components of glutamatergic neurotransmission. In addition, these neuron-like cells responded to stimulation with glutamate with rapid and synchronised calcium influx [102].

Before considering stem cell transplantation in clinical trials, it would be necessary to design culturing condition free of animal products to reduce the possibility of contamination and possible infections. To avoid any effect an undefined population of serum proteins might have on differentiation pathways, a serum-free media should be optimized. It is also important to keep the *in vitro* culturing time as short as possible to reduce the risk of infections and chromosomal aberrations seen in other stem cells. Serum-free culturing conditions for harvesting, expansion, neuronal differentiation and maturation of cord blood stem cells without stimulating the hematopoietic commitment of cord blood stem cells have been optimised [63] and when they were applied to cord blood stem cells in 3-dimentional scaffolds the differentiated cells were equally distributed inside the scaffolds and expressed mature neuronal specific markers, such as NF-200, β-tubulin, neural nuclei specific markers and post-synaptic density protein-95 [103].

Thus it appears that different populations of stem cells in umbilical cord blood, such as embryonic-like stem cells [60] MSCs [100,104,105] and CD34- /CD45- non-hematopoietic stem cells [94,100,106] may have the potential of differentiating into neural cells. The diversity in the reported methods of differentiation reflects the uncertainty in the precise identity of the cord blood cells that might differentiate into a neuronal- or glial-like phenotype, and it is uncertain whether a primitive multipotent stem cell resides in cord blood or whether a transdifferentiation process is responsible for neuronal differentiation from hematopoietic lineage [104]. Furthermore, claims that MSCs, in particular, are able to differentiate into neurons are disputed, with more recent studies reporting that limited transdifferentiation into astrocytes only is possible [108,109].

4. Experimental models of CNS repair using stem cells

4.1 Repairing adult stroke lesions using cord blood derived stem cells

In the animal models of adult stroke, umbilical cord blood and its derived cell populations have already been demonstrated to have therapeutic effects. Middle cerebral artery occlusion (MCAO) in rats has been used to mimic the progressive degenerative lesions following an embolic stroke in human, accompanied by robust immune cell recruitment in the striatum. In 2001, Chen *et al*. first demonstrated that intravenous administration of human umbilical cord blood ameliorated many of the physical and behavioural deficits in adult rats suffering MCAO [110]. Cord blood cells were shown to migrate into the ischemic area, attracted by cytokines and chemokines released in the ischemic tissue [111]. The therapeutic beneficial effects of cord blood transplants appear to be dependent on the timing of transplantation after injury [111], the cord blood cell dose [112] and the method of cell transplantation [113]. Intravenous infusion was suggested to be more effective than direct delivery of cells into the injury site [113] in producing long-term functional benefits and the therapeutic efficacy of cell transplantation was demonstrated even when cells were infused 48 hours after the lesion [111].

In order to create a more clinically relevant stroke model, spontaneous hypertensive rats were exposed to transient middle cerebral artery occlusion and treated with intravenous transplantation of human umbilical cord blood (HUCB) CD34+ cells transfected to express both elevated glial cell line-derived neurotrophic factor (GDNF) and green fluorescent protein. At 28 days after transplantation of GDNF gene modified CD34+ cells, significantly more green fluorescent protein (GFP) positive cells, including neurons and astrocytes, derived from the grafted cells, populated the peri-infarct area compared to those injected with GFP only CD34+ cells or vehicle. Furthermore, the stroke animals transplanted with GDNF gene modified CD34+ cells showed a significant increase in GDNF levels in the infarcted hemisphere, reduced brain infarction volume, and enhanced functional recovery compared with those that received GFP only CD34+ cells. This study supports the use of a combined gene and stem cell therapy for treating stroke [114].

These approaches have also been extended to other species. HUCB-derived MSCs have been delivered through the basilar artery in a canine thromboembolic brain ischemia model. Upon neurobehavioral examination, earlier

recovery was observed in HUCBC treated animals than in controls. The HUCBC treated animals also showed a decrease, rather than increase, in the infarction volume at 1 week after cerebral ischemic induction. It was claimed that transplanted cells had differentiated into neurons and astrocytes, whilst undifferentiated HUCB-derived MSCs expressed neuroprotective factors, such as BDNF and vascular endothelial growth factor (VEGF), at 4 weeks after the transplantation [115].

However, not all experimental studies have reported such positive results. Zawadska *et al.* intravenously infused HUCB cells at various time points after a transient middle cerebral artery occlusion in adult rats. Only a few human cells were found, localised to the ischemic area, mostly around blood vessels. Timing of HUCBC delivery after ischemia or injection of Cyclosporin A at the time of delivery had no effect on the number of human cells detected in the ischemic brain. Infusion of HUCBC did not reduce infarct volume and did not improve neurologic deficits after middle cerebral artery occlusion [116]. In spontaneously hypertensive rats, intravenous infusion of HUCB cells following permanent MCAO failed to reduce the volume of the infarct or reduce the expression of the apoptosis marker caspase 3 [117]. The authors questioned whether spontaneously hypertensive rats react in the same way to stroke and subsequent treatment as normal rats, which is an important consideration as the human population who suffer stroke are like to be in some way pre-disposed to suffering stroke, through being hypertensive or from some other cause.

4.2 Models of perinatal brain damage

Researchers have investigated the therapeutic potential of cord blood in models of perinatal brain damage as well. Meier and others [118] transplanted human cord blood mononuclear cells intraperitoneally 24 hours after left carotid artery ligation and 8% O_2 inhalation for 80 minutes on postnatal day 7. On postnatal day 21, the injected stem cells were found incorporated around the lesion without obvious signs of transplantation and the spastic paresis was largely alleviated, resulting in a normal walking behaviour, as assessed by footprint and walking pattern analysis. In a rat model of H/I injury at postnatal day 7, intraperitoneal transplantation of HUCB mononuclear cells, 3 hours after the H/I insult, resulted in better performance in two tests of developmental sensorimotor reflexes in the first week after the injury, along with a neuroprotective effect in the striatum and a decrease in the number of activated microglial cells in the cerebral cortex of treated animals [119]. Similar effects were found with intracerebral transplantation of umbilical cord blood MSCs into neonate rats with H/I brain injury [120]. A recent study has transplanted human MSCs by intracardiac injection into a rat model of neonatal H/I brain injury and shown improvements in motor performance [121].

MSCs from human umbilical cord blood of full-term newborns were isolated and intra-cerebrally transplanted into rat neonates after H/I induction. Immunostaining showed the transplanted MSCs migrated to the hippocampus. Rats receiving MSCs showed significant improvement in neurological function and alleviated brain tissue injury when compared with controls. Differentiation of MSCs into astrocytes, but not neurons, was observed [122]. MSCs from rat neonatal bone marrow (to mimic the phenotype of umbilical cord blood) have also been transplanted to a rat model of neonatal PVWMI soon after lesioning and been shown to reduce the extent of the failure of myelination, and partially rescue development of corticospinal motor function [123]. However, as with all these studies, it was unclear exactly what contribution the transplanted cells made to the recovery.

All the experiments described in this section made use of rodent models involving a perinatal injury induced between post-natal days 5 and 7, which can be considered as approximately equivalent to the beginning of the third trimester in humans [17] and thus most appropriate for modelling PVWMI [124, section 1.2]. Rodent experiments that have modelled stroke or asphyxiation at a stage of cortical development equivalent to term in humans are rare, as are experiments in primates in which the relative complexity of the subplate might more accurately reflect the situation in human development [42].

4.3 Transplantation of cord blood/ bone marrow derived stem cells in human

Early stage clinical trials using autologous transplantation of bone marrow derived cells, also present in umbilical cord blood, to test a variety of clinical conditions have been reported that have concentrated on the feasibility and safety of the approach. These include intraarterial transplantation of mononuclear cells in patients with middle cerebral artery ischemic stroke [125] intrathecal injection of MSCs in amyotrophic lateral sclerosis [126,127] and multiple sclerosis [126]. All these studies reported no serious adverse effects upon patients and no unexpected worsening of their neurological condition. However, efficacy of the treatment, as yet, has not been reported on.

The application of cord blood and derived stem cell populations in spinal cord injuries has shown promising results. Both transplanted human cord blood whole fraction and derived CD34+ cells were recruited into the site of injury of experimental rats and an improved functional recovery was observed [128,129]. Furthermore, a 37 year old female patient with spinal cord injury showed improved sensory perception and mobility, both functionally and morphologically, after the transplantation of human cord blood purified MSCs into the injured location. Computed tomography and MRI results showed the regeneration of the spinal cord at the injured site and some of the cauda equina below it [130].

4.4 Mechanisms of action

Transplantation of non-neural stem cells such as MSCs, consistently show functional improvements in animal models without evidence of the stem cells differentiating into neural cells, being incorporated into neural circuitry or even surviving in the brain [124,131,132]. For instance, it has been demonstrated that intravenous injection of bone marrow derived MSCs in an adult rat stroke model either 1 or 3 days post-lesion decreased post-operation mortality and enhanced both behavioural and neurological recovery. This was correlated with decreased glial scarring, reduced infarct size, increased revascularisation of the lesion site, and

increased cell division in the subventricular zone [133]. This suggests that the major benefits of cord blood cell transplantation may be not mediated by cell replacement, but by other mechanisms, for instance by reducing inflammation [134]. Reduced granulocyte and monocyte infiltration and a lack of astrocytic and microglial activation have been observed in the parenchyma [111]. Transplantation of cord blood-MSCs and cord blood-CD34+ cells directly into the brain parenchyma with ischemic damage increased cell survival and promoted an environment conducive to neovascularisation of ischemic brain which, in turn, lead to an increased sprouting of nerve fibres from the non-damaged hemisphere into the ischemically damaged side of the brain [120].

In another study, cord blood MSCs migrated towards the ischemic boundary zone and promoted the formation of new blood vessels, suggesting an increase of local cortical blood flow in the ischemic area [135]. A follow up to this study demonstrated that infused cord blood-MSCs were found around endothelial cells and expressed neuroprotective factors, such as BDNF, VEGF at four weeks after transplantation [115]. In a study in which seven-day-old Sprague-Dawley rats were subjected to unilateral HI, behavioural tests at post-transplantation showed that HI animals that received HUCB cells alone or when combined with mannitol were significantly less impaired in motor asymmetry and motor co-ordination compared with those that received vehicle alone or mannitol alone. Improvements in function were correlated with elevated levels of GDNF, NGF and BDNF in brain tissues. However, histological assays revealed only sporadic detection of HUCB cells, suggesting that the trophic factor-mediated mechanism, rather than cell replacement *per se*, principally contributed to the behavioural improvement [136]. In a tissue and cell culture model of cerebral ischemia, the release of antioxidants, the decrease of free radicals and the accumulation of growth factors were found in the media containing cord blood progenitors, all of which might confer neuroprotection [92].

However, caution may be needed when contemplating stem cell transplants to brain lesions that show a local inflammatory response, as is seen in PVWMI. Bone marrow stromal cells were administered into the cerebral ventricles of rats exhibiting experimental autoimmune encephalomyelitis, a model for multiple sclerosis, and migrated into the brain parenchyma forming cellular masses characterized by focal inflammation, demyelination, axonal loss and increased collagen-fibronectin deposition. Stem cells entering the inflamed central nervous system may be tumourigenic under certain conditions [137].

4.5 Could cord blood stem cells replace missing neurons?

Even if we accept that certain classes of cord blood stem cells, particularly embryonic stem cell like cells, do have neurogenic potential [102] would it still be feasible for transplanted cells to develop into corticospinal neurons and send long range projections to the spinal cord? Attempts to regenerate corticospinal axons in animal models of spinal cord injury have met with limited success due to the presence of limiting environmental factors such as growth inhibiting myelin and extracellular matrix, and reduced expression of trophic and tropic factors [138-140]. However, in the developing brain, such barriers may be less of a problem as myelination of the corticospinal tract is not completed in the infant [141] the extracellular matrix may not be mature and could still promote axon plasticity [142] and expression of growth promoting molecules may still be upregulated in this immature condition [143]. In addition, the corticospinal tract will simply not be as long reducing the time required for regeneration. This brings us back to the original proposal that the earlier such interventions are made, the better the chances of repair. Animal experiments have also demonstrated that reconstitution of long distance projections is more than a theoretical possibility. In rats where the corticospinal tract has been selectively ablated, endogenous stem cells migrate to the motor cortex, differentiate and send axonal projections to the spinal cord, as demonstrated by double labelling by retrograde tracing and birth-date determination [144]. Thus, the objective of repairing the corticospinal tract, either by indirectly stimulating endogenous stem cells with stem cell transplants, or by

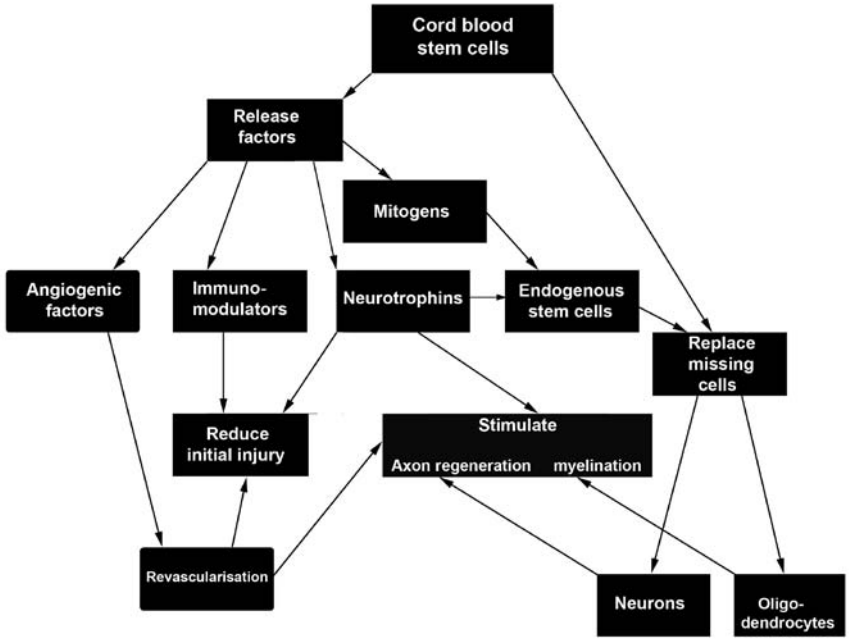

Figure 2. Pathways to brain repair. Intravenous or intracerebrally injected cord blood stem cells might be expected to accumulate at sites of injury where they may release various factors that could have a protective or reparative effect. Additionally, some cells may differentiate and directly replace missing cell populations.

stem cells differentiating into corticospinal neurons themselves, remains a possibility. Work underway to explore the differentiation pathways of human corticospinal neurons [145,146] may give insights into how to promote the differentiation of stem cells, endogenous and grafted, and bring about repair of the corticospinal tract.

5. Conclusion

Although many issues still remain to be resolved, the use of autologous cord blood stem cell transplantation has the potential to be a safe and effective treatment option, alongside other therapies, and gives hope that we could actually treat, rather than manage, the symptoms of cerebral palsy (Figure 2). The treatment might work best if administered early in the condition, before aberrant plasticity has taken hold, but this requires better understanding of the early stages of the condition and greater power to predict outcomes. Thus development of diagnostic procedures such as imaging and neurophysiological investigation must proceed hand in hand with advances in stem cell differentiation and transplantation, if a coherent programme of treatment is going to be developed.

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