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Editorial

Commentary – Exosomes: Realization of the great therapeutic potential of stem cells

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1. Introduction

Interventions in neonatal brain injury are best considered as neuroprotective or neurorestorative or both. In general, neuroprotective interventions prevent primary cellular injury or death, and neurorestorative interventions ameliorate or prevent the subsequent disturbances of brain maturation, i.e., hypomyelination, impaired neuronal development, altered connectivity, etc. The purposes of this commentary is to review briefly the neuroprotective and neurorestorative potential of stem cells, but particularly to discuss the recent evidence that stem cell-derived exosomes are the principal mediators of these beneficial effects and that these small vesicles could prove to realize the potential of stem cell therapy in perinatal brain disease.

2. Stem cells

Experimental studies of perinatal hypoxic-ischemic disease, stroke, white matter injury, and intraventricular hemorrhage in neonatal animals indicate that *stem cells* have both neuroprotective and neurorestorative properties [1–17]. The cells have shown benefit after administration in the acute period (neuroprotective) and in the chronic phase (neurorestorative). Multiple stem cell populations have been studied but mesenchymal stem cells (MSCs), especially from human umbilical cord blood (or Wharton's jelly) (UCB), have been used particularly commonly. MSCs have been favored because of relative effectiveness, ready availability and low immunogenicity. A variety of routes of cell administration have been utilized. Intracerebral, intraventricular, intravenous, and intranasal approaches have been most common. Intravenous delivery has resulted in definite, though variable benefit in experimental models [9, 16, 18]. Notably, systemic administration of MSCs results in substantial retention in lungs and other systemic organs [19]. ***Intranasal*** administration has been especially efficient and effective [1, 4, 7, 15, 17]. (Stem cells administered by this route appear to target the injury site after entering the brain via olfactory neural processes traversing the cribriform plate). However, several factors have hindered broad use of stem cells in newborn infants with neurological disease, e.g., nonoptimal routes for repetitive administration, the possibility of tumorigenic potential, and immunogenicity, albeit low.

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Some of the concerns regarding stem cell therapy have been addressed by the use of autologous cord blood therapy. Such an approach has been shown to be feasible in human neonatal hypoxic-ischemic encephalopathy, and clinical trials are in progress [14, 20]. However, logistical issues related to collection, processing, storage, and dosing are substantial.

The balance of experimental data indicates that the beneficial effects of stem cells relate not principally to engraftment and differentiation of the stem cells but, rather, to paracrine effects, i.e., factors released by stem cells exert the beneficial effects [19]. The mediators of these paracrine effects, presumably, could constitute more direct and specific therapeutic agents. Although neurotrophins and other molecules may be involved, it now appears plausible that the principal mediator of the paracrine effects of stem cells is the exosome.

3. Exosomes

Exosomes are a type of small (20–100 nm) membrane bound vesicle secreted from all cells in both physiological and pathophysiological states [21–24]. They carry proteins, lipids and nucleic acids, including various RNA species, particularly microRNA (miRNA). These vesicles are involved in cell-cell communication, and in brain, in communication between brain cells (neurons and glia) as well as between these cells and the periphery [23]. One excellent example of these communications within the developing brain involves the coordination by oligodendrocyte exosomes of myelination of axons by oligodendrocytes [23]. Importantly, exosomes can cross the blood-brain barrier in either direction, thus allowing analysis of blood to assess the status or function of specific brain cells and entry into brain from blood for neuroprotective or neurorestorative functions. The latter will be emphasized in this Commentary. A notable example of the former role is detection in blood of exosomes that contain Alzheimer disease-specific oligomeric proteins and thereby providing the potential to provide ultrasensitive early detection of Alzheimer disease [25]. Concerning the latter role, increasing evidence indicates that exosomes from MSCs mediate the beneficial effects of stem cell therapy in various pathological neurological states, including perinatal brain injury (see later). Particular promise for MSC-derived exosomes in neonatal disorders has already been shown in experimental models of necrotizing enterocolitis [26] and bronchopulmonary dysplasia [27].

Of the cargo in exosomes, miRNA is of major importance in mediating therapeutic effects (see later). Importantly, MSC-derived exosomes are readily isolated and can be engineered to contain selected miRNA species to enhance the therapeutic effects in neuropathological states (see later). Exosomes derived from UCB-derived MSCs have low immunogenicity, high biocompatibility, and strong targeting ability [22]. They maintain high activity during relatively long-term storage, can be administered intravenously and, as noted earlier, readily cross the blood-brain barrier [23].

4. Exosomes and brain injury

Exosomes derived principally from MSCs have been shown to exhibit both neuroprotective and neurorestorative properties. The experimental models have included studies focused on mature and on developing animal models. In the following, I will emphasize the latter.

Experimental models *of brain injury of mature animals* have included stroke, intracerebral hemorrhage, spinal cord ischemia, and traumatic brain injury [28–35]. Exosomes were prepared from MSCs from multiple sources. The exosomes were administered usually intravenously, but occasionally by intrathecal or intranasal routes. Both neuroprotective and neurorestorative effects were observed. The former consisted principally of prevention of neuronal death and neuroinflammation. A particularly informative recent example involved intravenous infusion of exosomes in the hours after stroke, induced in adult rats by middle cerebral artery occlusion/reperfusion [34]. The exosomes led to a reversal of microglial polarization from a damaging M1 state to a protective M2 state. Microglial pro-inflammatory cytokines were diminished, anti-inflammatory cytokines and neuroprotective factors were increased, and neurological outcome was improved. In view of the critical role of microglial-mediated inflammation in the genesis of perinatal brain injury [36], the findings are of major interest. Later neurorestorative effects in adult models have involved axonal outgrowth, oligodendrogenesis, repair of white matter, angiogenesis, and improved neurological function subsequently. Although comparisons of effectiveness are difficult, one head to head comparison of treatment with MSCs versus exosomes in a stroke model showed equal

improvement with both approaches [37]. Moreover, engineered exosomes (see later) containing selected miRNA had more potent therapeutic effects in stroke and traumatic brain injury than did naive MSC-derived exosomes [23].

Experimental models of *perinatal brain injury* have emphasized studies of hypoxic-ischemic and inflammation-induced injury, especially to cerebral white matter. The reports have appeared in the past five years [38–41]. Concerning perinatal *hypoxic-ischemic injury* alone, Ophelders et al. studied mid-term ovine fetuses subjected to transient umbilical cord occlusion [38]. Exosomesderived from MSCs (prepared from bone marrow) were administered intravenously one hour and four days following the insult. The intervention led to reduced seizures and partial prevention of hypomyelination.

A later study in a developing animal model assessed the *inflammatory brain injury* induced by lipopolysaccharide injected on P3 in rats [39]. Exosomes derived from bone marrow MSCs were injected intraperitonially at three hours before and 24 hours after the lipopolysaccharide. The beneficial effects in brain were prominent, i.e., prevention of reactive gliosis at P4, decrease of cell death in cortex and white matter at P5, amelioration of hypomyelination at P11, improved cognition at P30 and P90, and restored microstructure of white matter by MRI (increased fractional anisotropy and reduced radial diffusivity) at P125.

The most recent studies in developing animal models have focused on the use of exosomes by the intranasal route for *inflammation-induced and combined hypoxia-ischemia/inflammation-induced perinatal brain injury* [40, 41]. In both *in vitro* and *in vivo* (P3 rats) experiments, intranasal exosomes sharply reduced microglia-mediated neuroinflammation [41], key mediator of cerebral white matter injury in premature infants [42]. In a subsequent, still more relevant study, the same group assessed the effects of intranasal exosomes (prepared from UCB-MSCs) in a model of hypoxia-ischemia (carotid occlusion) and inflammation (lipopolysaccharide injection) — induced brain injury in P2 rats [40]. By using infrared-labeled exosomes and *in vivo* imaging, it could be shown that intranasally administered exosomes reached the frontal regions within 30 minutes after administration and distributed throughout the brain after three hours. The therapy led to reduced neuronal death in the subplate zone of parietal cortex and in the hippocampus. Moreover, an increased number of mature oligodendrocytes and normal myelination was observed at PH. At four weeks, improved neurological outcome was observed in the exosome-treated animals.

Taken together, the studies of perinatal brain injury support that systemic or intranasal delivery of exosomes have both neuroprotective and neurorestorative properties. The findings suggest further that a cell-free preparation of MSC-derived exosomes can substitute for the cellular counterpart in the treatment of newborns, especially perhaps preterm newborns with hypoxic-ischemic and inflammation-induced brain injury.

5. Engineering exosomes —how to make a good treatment even better

Therapeutic benefits of exosomes could be enhanced by altering its cargo to serve specific purposes. Exosomes are enriched in miRNAs and *in vivo* and *in vitro* studies specifically support that the therapeutic effects of MSC-derived exosomes can be attributed largely to the miRNA cargo (although delivery of cargo proteins can also play a role in some instances) [23]. This feature underlies the possibility tailoring exosomes to target specific recipient cells in the brain more efficiently than with naive cell-derived exosomes. Thus, exosomes can be engineered to target delivery of miRNA to brain cells, according to the disorder to be treated. For example, in a model of traumatic brain injury, miRNA-enriched exosomes led to polarization of microglia to a non-inflammatory phenotype, enhanced neurogenesis and improved functional recovery [32]. As noted earlier, pro-inflammatory microglia are important mediators of cerebral white matter injury in premature infants [36]. Moreover, tailoring of exosomes could enable multiple targets to be targeted at different times after a neural insult. Exosomes loaded with a neuroprotective miRNA could be administered in the acute period, and exosomes loaded with a restorative miRNA could be administered during the subacute and chronic phases [23].

6. Conclusions

Although stem cells have been shown to convey benefit in a variety of experimental models of perinatal brain injury and in some human disorders, difficulties with routes of administration for repetitive administration, preparation, and safety have been

considerable. Moreover, the discovery that the beneficial effects of stem cells are mediated principally by paracrine effects has led to the search for the mediators of benefit. Exosomes, small vesicles released by stem cells and capable of crossing the blood-brain barrier, appear to be principal mediators of benefit. Recent experimental studies support this conclusion and tailored exosomes can enhance the benefit. Scaling up of exosome isolation and production for use in humans now seems achievable and lyophilization can be used to preserve exosomal cargo bioavailability [23]. Although safety considerations need full exploration, the neuroprotective and neurorestorative properties of exosomes appear to present the possibility for a sea change in the management of the infant with neurological injury.

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