Congenital-neonatal hydrocephalus: therapeutic alternatives to derivation. A look at cell therapy

Hidrocefalia congénita-neonatal: alternativas terapéuticas a la derivación. Una mirada a la terapia celular

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Abstract

Introduction: Fetal hydrocephalus, perinatal and neonatal represents a major therapeutic challenge and often has with poor neurological prognosis, due to its heterogeneous aetiology, including neurodevelopmental disorders. **Objective:** To make a collection of advances in cell therapy as a proposal that can extend the spectrum of treatment in congenita-neonatal hydrocephalus. **Development:** Therapeutic interventions available at present as the ventricle-peritoneal shunt and third ventriculotomy, are insufficient to fully resolve the hydrocephalus and to prevent or reverse the associated neurological damage. This is why what has emerged the need for new therapeutic alternatives based on the knowledge of physiopathological mechanisms involved in the development of this condition. In particular, cell therapy with neural stem cells and mesenchymal stem cells has proven in animal studies and preclinical studies with humans, efficiently and safely to prevent hydrocephalus originated from the disruption of the ventricular zone and secondary to intraventricular hemorrhage, with the consequent prevention of neurological sequelae sensorimotor and cognitive. **Conclusions:** So far, we do not have an efficient treatment that offers quality of life to patients with hydrocephalus, and that alternative therapy to be effective.

Key words: Congenital hydrocephalus. Hydrocephalus neonatal. Cell therapy. Intraventricular hemorrhage. Ventriculoperitoneal derivation. Stem cell.

Resumen

Introducción: La hidrocefalia de inicio fetal, perinatal y neonatal representa un gran reto terapéutico y a menudo cursa con un pronóstico neurológico pobre, debido a su etiología heterogénea, que incluye alteraciones del neurodesarrollo. **Objetivo:** Realizar una recopilación de los avances en terapia celular como propuesta que permite ampliar el espectro de tratamiento en la hidrocefalia congénita-neonatal. **Desarrollo:** Las intervenciones terapéuticas disponibles actualmente, como la derivación ventrículo-peritoneal y la tercera ventriculostomía, son insuficientes para resolver por completo la hidrocefalia y para prevenir o revertir los daños neurológicos asociados. Es por esto por lo que ha surgido la necesidad de crear nuevas alternativas terapéuticas a partir del conocimiento de los mecanismos fisiopatológicos que participan en el desarrollo de esta condición. Particularmente, la terapia celular con células madre neuronales y células madre mesenquimales ha demostrado,

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en estudios con animales y en estudios preclínicos con humanos, ser eficiente y segura para prevenir la hidrocefalia originada a partir de la disrupción de la zona ventricular y secundaria a la hemorragia intraventricular, con la consiguiente prevención de las secuelas neurológicas sensoriomotoras y cognitivas. **Conclusiones:** Hasta el momento no tenemos un tratamiento eficiente que ofrezca calidad de vida a los pacientes con hidrocefalia, y que esa alternativa terapéutica sea efectiva.

Palabras clave: Hidrocefalia congénita. Hidrocefalia neonatal. Terapia celular. Hemorragia intraventricular. Derivación ventriculoperitoneal. Células madre.

Introduction

Congenital and neonatal hydrocephalus is a pediatric condition of heterogeneous etiology, whose causes include excessive cerebrospinal fluid (CSF) production or obstruction of its circulation due to neurodevelopmental alterations¹. On the other hand, there is hydrocephalus secondary to injuries triggered by intracranial hemorrhage, brain infections or tumors, which most often occur during neonatal period².

Some studies suggest that up to 78% of patients with congenital or neonatal hydrocephalus suffer from residual neurological deficits, with disability rates that reach to up to 28%³.

Currently, there are three therapeutic options for fetal and neonatal hydrocephalus: ventriculoperitoneal shunt appears as the first option, which is indicated in neonates with body weight of more than 2000 g, with extraventricular intermittent drainage being the alternative in patients with low body weight until they reach the goal for surgical intervention; as a third option there is third endoscopic ventriculostomy, whose indication is limited to non-communicating-type hydrocephalus and in patients older than 1 year^{1,4,5}.

From 80 to 90% of neurological deterioration suffered by neonates with fetal-onset shunt-dependent hydrocephalus is not reversed by surgery⁶. Evidently, available therapies for fetal- and neonatal-onset hydrocephalus are insufficient to provide a real solution to this condition and to improve these patients' neurological prognosis and, in addition, they represent high costs for the health system in the long-term scenario.

The purpose of this review is to present the investigations available on these treatments and the findings that have been described.

Congenital-neonatal hydrocephalus pathophysiology

Congenital and neonatal hydrocephalus can be caused by primary genetic anomalies that directly

develop the damage, or by secondary injury mechanisms that occur as a result of ventricular expansion or altered CSF physiology. Recent studies suggest that, in addition to malformations such as Chiari II and Dandy-Walker, the main congenital mechanisms involve stenosis or obstruction of the Sylvian aqueduct, ependymal denudation and subcomisural organ (SCO) alterations³.

As for secondary injury mechanisms, intraventricular hemorrhage (IVH) originating in hemorrhage of the germinal matrix appears as the main cause.

Hydrocephalus originating in disruption of the ventricular zone

There are two germinal areas, known as ventricular zone (VZ) and subventricular zone (SVZ), which are associated with the ventricular wall and where all brain cells are produced. The VZ is made up of radial neuroglia or neural stem cells (NSC), which are multipotent cells differentiated from the neuroepithelial cells that cover the neural tube until week 12 of gestation. The SVZ emerges around week 14, when NSCs are divided originating the neural progenitor cells (NPC), on whose proliferation depends the formation of this second germinal zone. From about 18 weeks of gestation on, NPCs are differentiated into neuroblasts and initiate neuronal migration using NSCs basal process as anchorage. In turn, the process of ependymogenesis (differentiation of radial neurons/NSCs into ependymal cells to line the ventricular walls) begins at around 18 weeks and is completed after birth, while gliogenesis starts at around 15 weeks and continues for several months after birth⁷.

Sylvian aqueduct of VZ interruption is associated with the appearance of hydrocephalus^{8,9}. This alteration arises from a common process that includes alterations in the traffic of vesicles, abnormal cell bonds and VZ integrity loss. This process starts around week 16 and ends during the first postnatal week, equally affecting the NSC and the ependymal cells that form

the VZ in the prenatal and postnatal stages of development, respectively¹⁰.

Until week 12 of gestation, in the neural tube, neuroepithelial cells differentiate into NSC to form the VZ; these tight bonds stop being expressed and the adhesion of NSC cells occurs only by gap bonds and adherent bonds (dependent on calcium with N-cadherin)^{9,11}.

The causes of secondary hydrocephalus are: 1) loss of PTB, an RNA-binding protein expressed in NSCs, induces adherent bonds gradual lack of expression in VZ, with hydrocephalus onset¹²; 2) animals with Numb and Numbl protein loss develop hydrocephalus, with these proteins being necessary to maintain adherent bonds in NSCs¹³; 3) the use of antibodies against N-cadherin, the main molecule involved in adherent bonds, triggers the detachment of ependymal cells from Sylvian aqueduct dorsal wall, causing hydrocephalus¹⁴; and 4) hydrocephalus develops in mice with the mutation of a gene that codes for α Snap, which is an intracellular traffic protein whose alteration results in N-cadherin abnormal transport to NSCs plasma membrane, accumulating in the cytoplasm, with the consequent massive disruption of VZ¹⁰.

Ciliary beat is essential to maintain CSF laminar flow, ergo, VZ disruption with loss of some multiciliated ependymal areas affects the CSF path through the aqueduct, contributing to the progressive development of hydrocephalus, and complete loss of ependyma of the aqueduct dorsal wall leads to the fusion of the denuded ventral and dorsal walls, with subsequent stenosis and obliteration of the cerebral aqueduct, causing the appearance of hydrocephalus^{10,11}.

VZ disruption results in two significant neuropathological events: NSC/NPC translocation to the CSF, turning into neurospheres that show the same cell binding alteration characteristics and that can be used to study this phenomenon^{9,15}; and the formation of sub-ependymal gray matter periventricular heterotopia (PH), which corresponds to the accumulation of neuroblasts as a consequence of their failure to migrate due to the loss of the NSC structure that serves them as anchorage¹⁰. In the human hydrocephalic fetuses that have been studied, PH has been found in 21- and 40-week fetuses¹¹. PH behaves like epileptogenic foci and can explain the epilepsy that occurs in patients with congenital hydrocephalus, affecting 6-30% of cases^{11,16}.

In addition to abnormalities in cell bonds and vesicle transport, other mechanisms that cause VZ disruption should be taken into account, such as infections or intracerebral hemorrhage, and only in a minority of

cases it is associated with Mendelian inheritance, with X-linked hydrocephalus being the most common type⁸.

Hydrocephalus secondary to intraventricular hemorrhage

IVH occurs as a result of bleeding of the germinal matrix (GM), which is a structure located along the lateral ventricle that is highly vascularized. IVH pathogenesis has been associated with: 1) alteration in cerebral blood flow; 2) GM vasculature inherent fragility (caused by blood-brain barrier [BBB] immaturity, including pericyte scarcity); and 3) coagulation disorders⁶. These conditions, associated with clinical risk factors such as vaginal delivery, Apgar low score, severe respiratory distress syndrome, pneumothorax, hypoxia, hypercapnia, seizures and infection, have been suggested to be able to cause significant fluctuations in cerebral blood flow or in vascular blood pressure, thus contributing to microvasculature rupture with the consequent GM hemorrhage^{16,17}. After GM bleeding with drainage to cerebral ventricles, blood hemolysis in the intraventricular space elevates the concentration of extracellular hemoglobin, which initiates inflammatory, chemotactic and apoptotic responses¹⁸. This imbalance between CSF production and reabsorption results in post-hemorrhagic hydrocephalus occurrence¹⁹.

More than 50% of premature newborns with severe IVH (grade > 3) die or develop post-hemorrhagic hydrocephalus (PHH), which requires shunting surgery in up to 70% of cases¹⁷. IVH is associated with significant brain damage, especially in periventricular white matter, which is exacerbated by PHH and ultimately results in increased mortality and long-term neurological morbidity, such as seizures, cerebral palsy and developmental delay in survivors.

Available cell therapies for the treatment of hydrocephalus

Recent research has laid the groundwork for cell therapy as a therapeutic alternative for fetal and neonatal hydrocephalus. Cells that are potentially useful for these transplantations or grafts are NSCs, mesenchymal stem cells (MSC), induced pluripotent stem cells and SCO cells. The expected result with this therapy is a suitable microenvironment for the neurogenic embryonic niche and, consequently, a normal brain development⁶.

In animal models, NSC/NPC, whether free or forming neurospheres, are easy to obtain and are the most reliable candidates because of their function of forming neurons and glia, and due to their low risk of tumorigenesis. Despite technical and ethical limitations, NSCs have been obtained from human embryos and allografts in animal models of patients with Parkinson's disease^{20,21}. There is a variety of stem cells, such as human embryonic stem cells, MSC or induced pluripotent stem cells, which are under investigation for the treatment of Parkinson's disease and other human brain disorders^{22,23}.

Neural stem cells

Taking into account the already described pathophysiological mechanism of VZ disruption, grafts of neurospheres formed by NSC or NPC have been implanted in the lateral ventricles of rats with hydrocephalus, for regenerative purposes. Forty-eight hours after transplantation, grafted cells are selectively integrated in VZ areas where denudation was produced, and it is believed that the ability of NSCs to migrate and differentiate into the required cell type depends on damaged areas that release specific chemotactic factors¹⁰. In mice with autoimmune encephalomyelitis, where neurospheres grafted in CSF originate NPCs that enter the demyelinated areas, differentiate into mature brain cells and promote multifocal myelination and functional recovery²⁴.

There are at least five advantages of using NSCs: they are available in embryonic and adult brain, they can be transplanted, they migrate, they have the differentiation capacity and they integrate to damaged areas. NSCs in adult SVZ have the potential to be an autologous source of replacement cells. When the brain is injured, NSCs are activated, proliferate and differentiate into newly born cells, and integration of these cells can replace damaged neurons¹⁰. However, this protection mechanism requires certain conditions and has a limit; therefore, finding mechanisms that facilitate and enhance the benefits of these grafts has been necessary.

A study conducted by Yuan et al.²⁴ assessed the protective effect of the combination of endogenous NSCs mobilization by the granulocyte-colony stimulating factor (G-CSF) and treatment with lithium chloride in PHH. This study was based on several key concepts: 1) G-CSF stimulates the proliferation, survival and maturation of cells with affinity for the granulocyte lineage through binding to G-CSF-specific receptor;

2) lithium chloride is a neuroprotective agent that inhibits cell apoptosis and inflammation by suppression of glycogen synthase kinase 3 β activity and improvement of BrdU absorption; and 3) lithium also regulates Wnt signaling pathway, which plays a key role in NSC proliferation and differentiation. The results showed that individual use of G-CSF or lithium chloride only partially relieved the incidence of PHH. Conversely, combined treatment with G-CSF and lithium chloride significantly attenuated ($p = 0.015$) the development of hydrocephalus after IVH in rats. The results also showed that neuronal apoptosis was significantly reduced by G-CSF and lithium chloride combined treatment ($p = 0.001$)²⁴.

Regarding the optimal timing for NSC grafting, it should be remembered that VZ disruption occurs at around 16 weeks and continues during the second and third trimester of pregnancy; it seems then reasonable to suggest that NSC graft should be carried out shortly after this VZ denudation process has been activated. Fetal surgery to repair neural tube defects, such as myelomeningocele and spina bifida, is performed at between 19 and 25 weeks according to the MOMS study, with this being the range established by the size and fragility of the fetus before 18 weeks and because of the increased risk of preterm delivery after 30 weeks²⁵. It is important having in mind that these patients have VZ disruption and that most fetuses born with spina bifida have hydrocephalus^{10,25}. NSC graft in the hydrocephalic brain would result in cell replacement or generation of a protective microenvironment to avoid progressive VZ interruption and to improve favorable glial responses²⁵.

All these investigations with NSC need isolation of these cells of human origin, for which good advances have been made by obtaining a line of immortal NSCs²⁶ and a line of striatum-derived NSCs²⁷.

Mesenchymal stem cells

These cells are particularly attractive for cell therapies, since they are versatile and multipotent adult stem cells that are able to differentiate into various cell types, including NPCs⁶. Although bone marrow is the best characterized MSC source, its use is limited because it involves an invasive process and because of the low number of MSCs that are present¹⁹. There is great availability of these cells in umbilical cord blood, placental membranes and amniotic fluid, which are gestational tissues that are generally discarded in the hospital setting and, therefore, they have no

significant ethical or safety limitations^{19,28}. In addition, donors' older age has been shown to translate into a negative impact on MSCs expansion and differentiation potential, unlike of when they originate from gestational tissues, which show less immunogenicity, higher proliferation capacity, higher paracrine potency and higher therapeutic efficacy *in vivo*, in comparison with MSCs derived from adult tissues such as bone marrow¹⁹.

The usefulness of MSCs in the treatment of PHH was demonstrated in a study conducted in 2013 by Ahn et al.²⁹, who induced severe IVH (grade 3 or higher) in rats by injecting blood into the ventricles at fourth postnatal day. Subsequently, MSCs derived from umbilical cord blood or fibroblasts were intraventricularly transplanted on the sixth postnatal day. The results were evaluated by serial brain magnetic resonance tests, behavioral function tests (such as negative geotaxis and rotarod test) and histological and biochemical analyses in brain tissues and CSF obtained on postnatal day 32. Finally, the study demonstrated that intraventricular transplantation of MSCs derived from human umbilical cord blood, but not fibroblasts, was effective 2 days after severe IVH induction, significantly attenuating post-hemorrhagic hydrocephalus, altered behavioral function in negative geotaxis and rotarod tests, histological abnormalities such as increased astrocytic gliosis and TUNEL and GFAP-positive cells, increase of inflammatory cytokines (such as IL-1 α , IL-1 β , IL-6 and TNF- α), corpus callosum thickness reduction and MBP expression. MSCs protective effects could be mediated by their anti-inflammatory and anti-apoptotic paracrine effects, rather than by their regenerative abilities as in the case of NSCs. These effects may be mediated by various growth factors and cytokines, such as vascular endothelial growth factor, nerve growth factor, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor and IL-10, which also stimulate endogenous cell proliferation and differentiation after severe IVH; by secreting these trophic factors, MSCs could promote the change of a pro-inflammatory environment to an anti-inflammatory or tolerant environment²⁹.

The role of BDNF as a mediator of MSCs neuroprotective effect has been evaluated, and it has been concluded that this neurotrophic factor is key to the function of said cells to prevent PHH and attenuate neurological damage after IVH, since these effects were eliminated when BDNF neutralizing antibodies were administered by RNA transfer *in vitro* in a culture

of rat neuronal cells exposed to thrombin, and in turn these neuroprotective effects were restored by administering brain-derived neurotrophic factor (rhBDN) supplements³⁰.

MSC TRANSPLANTATION CONSIDERATIONS: TIME, ROUTE OF ADMINISTRATION AND DOSE

The correct timing for this cell therapy is a key issue, since it influences treatment efficacy. Park et al.³¹ carried out a study to assess the therapeutic window for umbilical cord blood-derived human MSCs transplantation after severe IVH, and found that MSC neuroprotective efficacy was time-dependent, with a significant attenuation of PHH and of the above-described brain damage markers, only when administered early, at 2 days of severe IVH development, but not with late grafting at 7 days.

The tested administration routes have been the intraventricular^{29,32,33}, intrathecal³⁴, intranasal^{35,36} and systemic intraperitoneal or intravenous routes³², to conclude that although both local intraventricular and intrathecal and systemic intravenous administration are effective by significantly attenuating PHH and brain damage criteria after severe IVH, the intraventricular route is preferable because it requires a dose five times lower than the intravenous route, and still it delivers a larger amount of MSCs to the periventricular lesion site³³. Furthermore, since anterior fontanelle is open in newborns, MSCs local transplantation is feasible in a clinical setting through a ventricular canthus, without any additional invasive operation¹⁹. MSCs injection into the ventricle results in the distribution of these cells around both periventricular zones, which indicates the successful delivery of transplanted MSCs to sites of inflammation or injury²⁹. However, given that more than 90% of IVHs occur within the first 3 postnatal days, a period of high vulnerability, some critically ill and unstable premature patients will not tolerate intraventricular administration; therefore, systemic intravenous administration is considered to likely be a better alternative in these patients³³. However, since the disadvantage in this scenario would be that systemically transplanted MSCs can be retained in other organs, such as the lungs, liver, spleen and kidneys^{19,33}, there is another route or mode of administration whose investigation is currently ongoing, and it is cell encapsulation technology, which is transplanted to the CSF and might overcome the problem of graft rejection. This strategy implies the use of

genetically-modified cells that secrete proteins with therapeutic potential and are immobilized within a polymeric semipermeable membrane, preferably of alginates as the most suitable material, due to their abundance and biocompatibility⁶.

Regarding the optimal dose, it has been observed that it can be reduced from 5x10⁵ to 1x10⁵ cells if local intraventricular administration is chosen instead of systemic intravenous administration^{19,37}.

MSC GRAFT SAFETY AND LONG-TERM RESULTS

The safety of MSC transplantation has been assessed in animal studies, where its application has been shown to be safe in the short and long term, without tumorigenicity appearing in any of them and with long-term neuroprotective effects in histological, sensorimotor and cognitive matters persisting^{19,36}. It was even demonstrated that less than 1% of grafted cells were detected 18 days after intraventricular administration³⁸.

Evidence has shown that the safety of stem cell therapies will depend on several factors, such as the state of differentiation and proliferative capacity of grafted cells, timing and route of administration, and long-term survival of the graft¹⁰. Evidence from pre-clinical studies indicating the long-term, maintained protective effects of MSC transplantation, without any adverse effect, justify the translation of MSC transplantation into clinical trials in humans for the treatment of neonatal brain disorders³⁷.

When assessing the safety and viability of intraventricular allogeneic transplantation of human umbilical cord blood-derived MSCs in premature newborns with severe IVH within 7 days after its detection, non-suspected death or anaphylactic shock was found within 6 hours after MSC transplantation, and death or hydrocephalus requiring shunting surgery at up to 1 year³⁹.

Subcomisural organ stem cells

SCO is an ependymal secretory gland located at the entrance of the cerebral aqueduct. Its main function is to secrete spondin to the CSF and form the Reissner fiber, which extends through the Sylvian aqueduct, the fourth ventricle and the central canal of the spinal cord, and it is indispensable for maintaining aqueduct permeability and normal CSF flow. SCO congenital defect results in hydrocephalus⁶. In addition to

spondin, SCO secretes transthyretin and the S100 β protein, which support embryonic brain development. Recently, evidence has been found to propose that these factors have similar functions in adult neurogenesis, regulating proliferation, migration and differentiation of NSCs and neuronal precursors in adult neurogenic niches. These findings provide a basis to motivate research for a therapy based on SCO cells.

Induced pluripotent stem cells

Currently, reprogramming adult skin fibroblasts in induced pluripotent stem cells is possible. This modality has been tested to cultivate three-dimensional neural tissue from human pluripotent stem cells and treat microcephaly⁴⁰. The resulting brain organoids reached up to 4 mm in size and contained polarized radial stem cells surrounding a cavity filled with fluid and that resembles the lateral ventricle in the developing brain. This model can serve as a valuable *in vitro* platform to study the molecular mechanisms that regulate the development of the brain cortex, and could provide an innovative and complementary approach to the study of the VZ disruption that leads to hydrocephalus and abnormal neuro/gliogenesis *in vitro*.

Conclusions

Cell therapy has emerged as a promising therapeutic alternative, and its importance goes beyond controlling hydrocephalus, focusing on improvement or even prevention of neurological sequelae that so far are considered irreversible. NSC and MSC transplantation efficacy to prevent congenital hydrocephalus by VZ disruption and neonatal post-hemorrhagic hydrocephalus, respectively, has been demonstrated. Its advantages include a once in a lifetime intervention, which would decrease complications arising from subsequent interventions and long-term costs; it has been proven useful to prevent and reverse neurological damage, which entails a lower morbidity load in patients with hydrocephalus; these cells are easily obtained, especially MSCs, and do not entail significant ethical limitations.

Some disadvantages are that, although the best time for NSC cell graft is at week 16, it is difficult to predict or diagnose at that stage of gestation whether the patient will develop hydrocephalus. Although most patients with spina bifida have been shown to develop hydrocephalus, there are no studies demonstrating this association and that allow assuming that every

patient with a neural tube defect will develop hydrocephalus, and thus justify transplantation. Umbilical cord blood is heterogeneous and the number of cells highly varies from one specimen to another, which makes dose standardization difficult.

So far, we don't have an efficient treatment that offers quality of life to patients with hydrocephalus, and that therapeutic alternative that we need will arise from a better understanding of the biological mechanisms of brain abnormalities that precipitate congenital and neonatal hydrocephalus.

Conflict of interests

None of the authors or their direct family members have conflicts of interest that may influence the content of this manuscript.

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