Experimental Neurology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

# Long-term hydrocephalus alters the cytoarchitecture of the adult subventricular zone

Tania Campos-Ordoñez [a], Vicente Herranz-Pérez [b,c], Kaisorn L. Chaichana [d], Jordina Rincon-Torroella [d], Daniele Rigamonti [d], Jose M. García-Verdugo [b,c], Alfredo Quiñones-Hinojosa [d,*], Oscar Gonzalez-Perez [a,**]

[a] Laboratory of Neuroscience, Facultad de Psicologia, University of Colima, 28040, Mexico
[b] Laboratory of Comparative Neurobiology, Instituto Cavanilles, University of Valencia, Valencia 46980, CIBERNED, Spain
[c] Unidad mixta de Esclerosis múltiple y neurorregeneración, IIS Hospital La Fe-UVEG, Valencia 46013, Spain
[d] Department of Neurosurgery, Johns Hopkins School of Medicine, Baltimore, MD 21201, USA

ARTICLE INFO

*Article history:*
Received 17 March 2014
Revised 14 April 2014
Accepted 10 May 2014
Available online xxxx

*Keywords:*
Subventricular zone
Neural stem cells
Hydrocephalus
Aqueductal stenosis
Neurogenesis
Fractones
Human brain

ABSTRACT

Hydrocephalus can develop secondarily to a disturbance in production, flow and/or absorption of cerebrospinal fluid. Experimental models of hydrocephalus, especially subacute and chronic hydrocephalus, are few and limited, and the effects of hydrocephalus on the subventricular zone are unclear. The aim of this study was to analyze the effects of long-term obstructive hydrocephalus on the subventricular zone, which is the neurogenic niche lining the lateral ventricles. We developed a new method to induce hydrocephalus by obstructing the aqueduct of Sylvius in the mouse brain, thus simulating aqueductal stenosis in humans. In 120-day-old rodents ($n = 18$ per group), the degree of ventricular dilatation and cellular composition of the subventricular zone were studied by immunofluorescence and transmission electron microscopy. In adult patients (age > 18 years), the sizes of the subventricular zone, corpus callosum, and internal capsule were analyzed by magnetic resonance images obtained from patients with and without aqueductal stenosis ($n = 25$ per group). Mice with 60-day hydrocephalus had a reduced number of Ki67+ and doublecortin + cells on immunofluorescence, as well as decreased number of neural progenitors and neuroblasts in the subventricular zone on electron microscopy analysis as compared to non-hydrocephalic mice. Remarkably, a number of extracellular matrix structures (fractones) contacting the ventricular lumen and blood vessels were also observed around the subventricular zone in mice with hydrocephalus. In humans, the widths of the subventricular zone, corpus callosum, and internal capsule in patients with aqueductal stenosis were significantly smaller than age and gender-matched patients without aqueductal stenosis. In summary, supratentorial hydrocephalus reduces the proliferation rate of neural progenitors and modifies the cytoarchitecture and extracellular matrix compounds of the subventricular zone. In humans, this similar process reduces the subventricular niche as well as the width of corpus callosum and internal capsule.





## Introduction

Hydrocephalus is a neurological condition produced by disturbances in the production, flow, and/or absorption of cerebrospinal fluid (CSF), which results in dilation of the ventricular system. According to its time course, hydrocephalus in humans can be classified as acute, subacute, or chronic. Acute hydrocephalus occurs over days, sub-acute hydrocephalus over weeks, and chronic hydrocephalus over months to years. Hydrocephalus has an estimated incidence of 1 in 1500 births and is associated with multifocal motor deteriorations and cognitive decline (Fernandez-Carrocera and Gonzalez-Mora, 2004; Gallia et al., 2006; Zhang et al., 2006). The management of hydrocephalus in both young and older patients remains important because CSF hydrodynamics play a key role in supporting neuronal function via a number of growth factors and setting the extracellular matrix milieu (Gonzalez-Perez et al., 2012; Tarnaris et al., 2006; Zhang et al., 2013). Disruption of these normal functions leads to various neurological disorders, which can impact one's cognitive function, motor abilities, and mental faculties (Moghekar et al., 2012; Tarnaris et al., 2009).

The subventricular zone (SVZ) is a neurogenic niche adjacent to the lateral wall of the lateral ventricles (Fuentealba et al., 2012). The ependymal cell layer, adjacent to the SVZ, propels CSF in the ventricular system (Mirzadeh et al., 2008; Sawamoto et al., 2006) and regulates brain development (Han and Alvarez-Buylla, 2010; Mirzadeh et al., 2010). In the adult brain, ependymal cells regulate cell lineage (Lim et al., 2000), as well as the proliferation and migration of precursor cells within the SVZ (Del Carmen Gomez-Roldan et al., 2008; Sawamoto

* Correspondence to: A. Quiñones-Hinojosa, Department of Neurosurgery, Johns Hopkins University, 1550 Orleans St, CRB-II, Room 247, Baltimore, MD 21201, USA. Fax: +1 410 502 7559.
** Correspondence to: O. Gonzalez-Perez, Laboratory of Neuroscience, Facultad de Psicologia, Universidad de Colima, Av. Universidad 333, Colima, COL 28040, Mexico.
*E-mail addresses:* aquinon2@jhmi.edu (A. Quiñones-Hinojosa), osglez@ucol.mx (O. Gonzalez-Perez).

http://dx.doi.org/10.1016/j.expneurol.2014.05.011
0014-4886/

et al., 2006). Recently, branched fractal structures (referred to as *fractones*) that resemble basement membranes but have a distinctive morphology have been described in the SVZ (Mercier et al., 2002, 2003). Fractones are extracellular matrix structures that are in direct contact with the adult neurogenic niche, and found to regulate the proliferation of neural stem cells by facilitating the infiltration of cytokines into the SVZ niche (Kerever et al., 2007).

Experimental models of hydrocephalus are not easy to establish because sudden obstruction of the ventricular system is often catastrophic (Gonzalez-Perez, 2012; Townsend et al., 2012). As a result, most models rely on genetic manipulation. Some studies utilize mutant animals lacking either cilia (Banizs et al., 2005; Chen et al., 1998; Sapiro et al., 2002) or have cilia with reduced motility (Ibanez-Tallon et al., 2004; Torikata et al., 1991). A study evaluating Tg737Orpk mice, which have a hypomorphic allele of Polaris that is an essential protein for ciliogenesis, showed that ciliary movement not only helps maintain CSF flow, but also directs tangential migration of neuroblasts born in the SVZ toward the olfactory bulb in mice with congenital hydrocephalus (Sawamoto et al., 2006). The chronic effects of obstructive hydrocephalus on the brain cytoarchitecture and neural progenitors are therefore difficult to establish because of a lack of non-genetic animal models. Herein, we describe a novel mouse model where we induce long-lasting hydrocephalus by pre-aqueductal obstruction. Our findings indicate that chronic hydrocephalus reduces neural progenitors in the rostral SVZ and alters fractone organization in the absence of severe reactive gliosis, tissue injury, and damage to epithelial cells. Hence, this experimental technique induces a primarily supratentorial hydrocephalus that is useful for studying the effects of increased hydrostatic pressure on the brain cytoarchitecture, extracellular matrix components, cellular apoptosis, cell migration, and the efficacy of pharmacologic and non-pharmacologic interventions on hydrocephalus.

## Materials and methods

### *Animals*

A total of 36 Balb/C male mice (P60) were used. Mice were assembled into two groups: sham-operated (control) and 60-d hydrocephalus groups. All animals were housed under standard biotery conditions in standard rearing cages (28 × 12 × 15 cm). The relative humidity in the mouse facility was between 40% and 60%. Temperature was maintained in an air-conditioned room at 22 ± 1 °C (the extreme room temperature was in a range of ±2 °C). This housing condition is within the norm dictated by international regulatory agencies. Nonetheless, it should be noted that a recent report suggests that this temperature range produces mild stress, which may be important when cancer or metabolic disorders are modeled in mice (Kokolus et al., 2013). Water and food were delivered ad libitum. All experimental procedures described herein were approved by the Institutional Animal Care and Use Committee of the University of Colima.

### *Surgical procedure in rodents*

Obstructive hydrocephalus was induced by placing a piece of cellulose acetate film into the atrium of the aqueduct of Sylvius. Mice were intraperitoneally anesthetized with 2% Avertin (2,2,2-tribromoethanol plus 2-methyl-2-butanol) pre-warmed to 37 °C. Then, animals were placed in a stereotaxic apparatus (RWD Life Science Co., Ltd Model 68003) with a heater pad to keep the animal's body temperature at 37 °C. The mouse's head was shaved and cleaned with 0.1% chlorhexidine gluconate solution, followed by 70% ethanol and 0.1% chlorhexidine gluconate. The skin was incised in the midline with a sterile surgical blade N15 from just anterior to Bregma to just posterior to Lambda. The pericranium was removed with a cotton swab. The coordinates used to access the atrium of the aqueduct were −2.6 mm (anterior–posterior) and 0 mm (medial–lateral) relative to Bregma. At that set point, a hole was drilled with pre-sterilized microdrills and fine forceps. In all cases, the deepest layer of the bone was carefully removed with forceps to expose the dura mater. Before introducing the occlusive film, the dura mater was opened with fine forceps. A piece of cellulose acetate film (3 × 0.5 × 0.1 mm; length, width and thickness, respectively) was then implanted into the ventricular system (Fig. 1A). To reduce bleeding during this process, absorbable gelatin sponges (Surgifoam, Ethicon™) were gently placed on the interhemispheric vein before introducing the cellulose acetate lamina. After introducing this film, one-minute compression was immediately applied to the blood vessel. The surgical wound was glued with tissue adhesive agent (3M™ Vetbond™ 1469SB). The sham procedure consisted of drilling a hole in the skull (2.6 mm and 0 mm (coordinates relative to Bregma)) and removing bone fragments without opening the dura matter. For anesthesia recovery, animals were placed in a pre-warmed cage containing a clean bed. Analgesia was provided with subcutaneous injections of ketorolac (Dolo-Vet™) (5 mg/kg) every 12 h for 1 day following surgery.

### *Morris water maze (MWM) task*

MWM is a widely used device to evaluate the presence of cognitive impairments. This experimental apparatus consisted of a circular water tank (120 cm wide and 40 cm high) surrounded by extra-maze cues. An escape platform (10 cm wide and 30 cm high) was placed in the water. To assess for gross physical, sensory, motor, or motivational impairments, we used the visible-platform version of the MWM (Gulinello et al., 2009; Vorhees and Williams, 2006). 53 days after surgery, 8 mice in the hydrocephalic group and 6 mice in the control group were used. The water temperature was 27 ± 1 °C that is sufficient to motivate the animals to escape, but insufficient to inhibit learning (Morris, 1984). All animals were tested in the morning between 9:00 and 11:00. Every day, all mice were randomly released into the water from one of the four quadrants. The location of the platform changed and mice were allowed to swim for 60 s to find the platform. After the animal found the platform, it was allowed to remain there for 60 s and was then moved to a pre-warmed cage. The time and distance needed to find the hidden platform were collected and analyzed. Data collection was automated by a video image motion analyzer (EthoVision, Noldus Information Technology, USA).

### *Tissue processing*

Mice were anesthetized with sodium pentobarbital (50 mg/kg body weight) before being sacrificed. Transcardial perfusion was done with 0.9% NaCl solution at 37 °C followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were post-fixed overnight at 4 °C in the same fixative. To analyze the complete organization of SVZ cells and the cytoplasmic transformation of astrocytes, 40-μm thick coronal sections were cut with a vibratome from 1.18 mm to −3.40 mm in the anterior to posterior direction relative to Bregma (Paxinos and Franklin, 2001). Fluorescent immunostainings were performed as described below.

### *Immunohistochemistry*

Samples were rinsed (10 min × 3) in 0.1 M buffer phosphate buffer saline (PBS) to rehydrate the tissue. The sections were then blocked in 0.1 M PBS + 0.1% Triton + 10% goat serum for 1 h at room temperature in agitation, and the samples were then incubated with primary antibodies overnight at 4 °C in blocking solution + 0.1% Triton-X. To label proliferating cells, a polyclonal rabbit IgG anti-Ki67 (1:500; Novocastra code: NCL-Ki67p) was used. To label neuroblasts, a polyclonal guinea pig anti-doublecortin (DCX) IgG (1:1000; Millipore, Cat. No. AB2253) was used. The following day, the tissue was left for 15 min at room temperature. The tissue sections were then rinsed three times with



Please cite this article as: Campos-Ordoñez, T., et al., Long-term hydrocephalus alters the cytoarchitecture of the adult subventricular zone, Exp. Neurol. (2014), http://dx.doi.org/10.1016/j.expneurol.2014.05.011