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Pathophysiology of congenital and neonatal hydrocephalus

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S U M M A R Y

The pathophysiology of congenital and neonatal hydrocephalus is not well understood although the prognosis for patients with this disorder is far from optimal. A major obstacle to advancing our knowledge of the causes of this disorder and the cellular responses that accompany it is the multifactorial nature of hydrocephalus. Not only is the epidemiology varied and complex, but the injury mechanisms are numerous and overlapping. Nevertheless, several conclusions can be made with certainty: the age of onset strongly influences the degree of impairment; injury severity is dependent on the magnitude and duration of ventriculomegaly; the primary targets are periventricular axons, myelin, and microvessels; cerebrovascular injury mechanisms are prominent; gliosis and neuroinflammation play major roles; some but not all changes are preventable by draining cerebrospinal fluid with shunts and third ventriculostomies; cellular plasticity and physiological compensation probably occur but this is a major under-studied area; and pharmacologic interventions are promising. Rat and mouse models have provided important insights into the pathogenesis of congenital and neonatal hydrocephalus. Ependymal denudation of the ventricular lining appears to affect the development of neural progenitors exposed to cerebrospinal fluid, and alterations of the subcommissural organ influence the patency of the cerebral aqueduct. Recently these impairments have been observed in patients with fetal-onset hydrocephalus, so experimental findings are beginning to be corroborated in humans. These correlations, coupled with advanced genetic manipulations in animals and successful pharmacologic interventions, support the view that improved treatments for congenital and neonatal hydrocephalus are on the horizon.

1. Introduction

Viewed in its simplest form, hydrocephalus occurs when cerebrospinal fluid (CSF) cannot be absorbed adequately, usually forcing the cerebral ventricles (and occasionally the subarachnoid spaces) of the brain to enlarge substantially. Whereas hydrocephalus can begin at any age, fetal, perinatal and neonatal onsets, with a reported incidence of 0.48 to 0.81 per 1000 live births,[1,2] are particularly difficult to treat and often result in the poorest neurological outcomes. Some studies suggest that up to 78% of patients with congenital or neonatal hydrocephalus suffer with residual neurological deficits,[2–5] others indicate that disability rates are decreasing and have now reached 28%.[6] No doubt a major factor in these poor outcomes is the extraordinarily high failure of the surgical treatments designed to shunt CSF from the ventricles to alternative absorption sites. Shunt malfunctions occur at the rate of 30–40% during the first year and exceed 50% during the second year of treatment.[7] In addition, the very early onset of ventriculomegaly in fetuses and neonates results in a protracted course of cellular damage that often is not reversible. The goal of this review is to summarize the cellular mechanisms that contribute to the pathophysiology of congenital and neonatal hydrocephalus and to identify some promising pharmacologic interventions that may improve outcome in the future.

2. Multifactorial nature of hydrocephalus

Congenital and neonatal hydrocephalus can be caused by a wide variety of developmental abnormalities or insults; the primary culprits are neural tube defects, infection, intraventricular hemorrhage, trauma, and tumors. In children, this condition is especially damaging because the expanding ventricles, accompanied by increasing CSF pressure, cause the flexible skull to enlarge; this in turn both compresses and stretches adjacent brain tissue.

It is important to recognize that the pathophysiology of congenital hydrocephalus almost always includes two separate mechanisms: primary genetic abnormalities that may affect outcome individually, and secondary injury mechanisms that occur mainly as a result of expanding ventricles and/or altered CSF physiology. An excellent review has recently summarized the genetic factors that contribute to congenital hydrocephalus.[8] This review will highlight some of the specific cellular consequences

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that accompany genetic abnormalities, but will also focus on the secondary effects of ventriculomegaly in the developing brain. Rodent studies, which have recently been corroborated by preliminary observations in humans, suggest that (in addition to gross malformations such as Chiari II and Dandy–Walker) the main congenital mechanisms involve aqueductal stenosis or obstruction, ependymal denudation, and alterations in the subcommissural organ (SCO).

Furthermore, the clinical presentation and course of hydrocephalus contain many features that contribute to the multifactorial nature of this disorder. Multiple etiologies and classifications exist – so many that the straightforward separation of 'communicating' (flow of CSF from the ventricular system to the subarachnoid spaces) and 'obstructive' (blockage of CSF flow anywhere within the cerebral ventricles) has been challenged.[9] This challenge promotes the concept that all ventriculomegaly is 'obstructive' in the sense that CSF absorption can be impaired by structural blockage or reduced physiological transport at the arachnoid membrane and its granulations, cranial nerve lymphatics, and capillaries of microvessels. Major variations can also occur in the different temporal and spatial progressions of hydrocephalus, and these differences could have important clinical consequences. For example, a relatively slow progression of ventriculomegaly over weeks and months may allow cellular plasticity to occur and thus promote intrinsic repair mechanisms. Likewise, preferential expansion of the occipital horns of the lateral ventricle may impact the optic radiations and thus cause selective visual deficits without involving locomotion and motor function. Regional effects such as this raise the caveat that much of our knowledge of the pathophysiology of hydrocephalus has been determined by examination of the cerebral cortex, probably because it is easily accessible and is more distorted than most other structures.[10] Consequently, extrapolations to other critical structures such as the hippocampus, basal ganglia, hypothalamus, and especially the cerebellum and brainstem, should be viewed with caution. The age of onset may influence outcome; e.g. perinatal induction should primarily affect neuronal differentiation rather than the neurogenesis that occurred in early gestation. Finally, although it is widely accepted that multiple shunt malfunctions play a major role in outcome, the specific effects of these repetitive insults have never been studied at the cellular level. Taken together, all of these variables highlight the multifactorial nature of hydrocephalus and make targeted studies particularly difficult.

3. Overlapping and interrelated injury mechanisms in hydrocephalus

Several reviews have discussed the many injury mechanisms known to occur in most types of hydrocephalus.[11–17] Briefly, the most acute mechanisms initiated hours to a few days after the onset of ventriculomegaly include compression and stretch of periventricular tissue, ischemia and hypoxia, and increased CSF pulsatility, most notably in the cerebral aqueduct. Additional mechanisms are recruited as ventriculomegaly becomes chronic and/or progresses to more severe forms: gliosis and neuroinflammation, periventricular edema, demyelination, axonal degeneration and slow axoplasmic transport, metabolic impairments, stagnant CSF flow, altered blood–brain barrier transport that can lead to toxicity as with reduced amyloid clearance, dendritic and synaptic deterioration resulting in altered connectivity, and cell death. The role of neuronal cell death in the overall pathophysiology of hydrocephalus is interesting because apoptosis and necrosis of cortical neurons seem to occur only after prolonged hydrocephalus, and while statistically significant reductions have been reported it may be that these changes are not biologically significant, since the total number of apoptotic neurons in the cerebral cortex is so low compared to all neurons in that region that the overall effect is probably negligible. By contrast, oligodendrocytes appear to be vulnerable during early stages of hydrocephalus and undergo significant apoptosis in the periventricular white matter. Thus, myelin formation in the developing hydrocephalic brain can be impeded by multiple simultaneous events: stretch, compression, interstitial edema, hypoxia, and oligodendrocyte death. Much work remains in order to determine the sequence of these events, their time course, and their ultimate contribution to neurological outcome.

4. Overview of brain damage in hydrocephalus

Although our understanding of the pathophysiology of congenital and neonatal hydrocephalus is far from complete and many critical questions remain unanswered, the collective evidence, obtained mostly from experimental studies, indicates that the following conclusions can be drawn at this time. (a) The age of onset strongly influences the degree of impairment. Whereas developing brains may be more capable of plasticity and recovery, overall the impact of in-utero or perinatal onsets usually predicts a worse neurological outcome. (b) Regardless of the injury mechanisms, the severity of the pathology is dependent on the magnitude and duration of ventriculomegaly. (c) The primary, or at least the earliest affected, targets are periventricular axons, myelin, and microvessels. (d) Secondary changes in neurons reflect responses to axonal disconnection, diminished cerebral blood flow and ischemia, and altered metabolism. (e) Cerebrovascular injury mechanisms are prominent (e.g. hypoxia, ischemia, capillary damage). (f) Gliosis and neuroinflammation play major roles in acute and chronic (subthreshold) injury. (g) Altered efflux of extracellular fluid, slow CSF flow, and altered capillary transport mechanisms cause accumulation of toxins. (h) Some but not all changes are preventable by draining CSF with ventricular shunts, extraventricular drains, and third ventriculostomy. (i) Considerable plasticity and compensation probably occurs, although this is a major area requiring further study; one example is the newfound lymphatic absorption of CSF that occurs adjacent to some cranial nerves as they exit the cranium. (j) Pharmacologic protection of the brain holds promise but more preclinical research is required.

5. Animal models of congenital and neonatal hydrocephalus

The H-Tx rat model is most often employed in experimental research. This strain develops congenital obstructive hydrocephalus following aqueductal stenosis in the late fetal and perinatal periods,[18–21] which correspond to the third trimester of human brain maturation. The hydrocephalic phenotype is characterized by four chromosomes within a heterozygous background[20] and incomplete penetrance.[22] Ventriculomegaly becomes severe by the second postnatal week (Fig. 1A) and animals will usually expire by 20–25 days of age if CSF is not drained with either ventriculo-peritoneal or ventriculo-subcutaneous shunts. Behavioral and cytopathologic studies clearly indicate that these shunts are more effective if placed early (3–5 days of age) rather than late (12–14 days of age) during the progression of ventriculomegaly. A few studies have taken advantage of the fact that about 10–15% of H-Tx offspring will only develop mild ventriculomegaly and will survive for months to years. The benefit of this chronic subtype within this strain is that the slowly progressive nature of hydrocephalus can be examined over a long period of time. Inbred strains of Wistar–Lewis (LEW/Jms) rats also develop aqueductal stenosis through non-Mendelian mechanisms as early as 4 days before birth,[23] and are excellent models for studying neonatal and

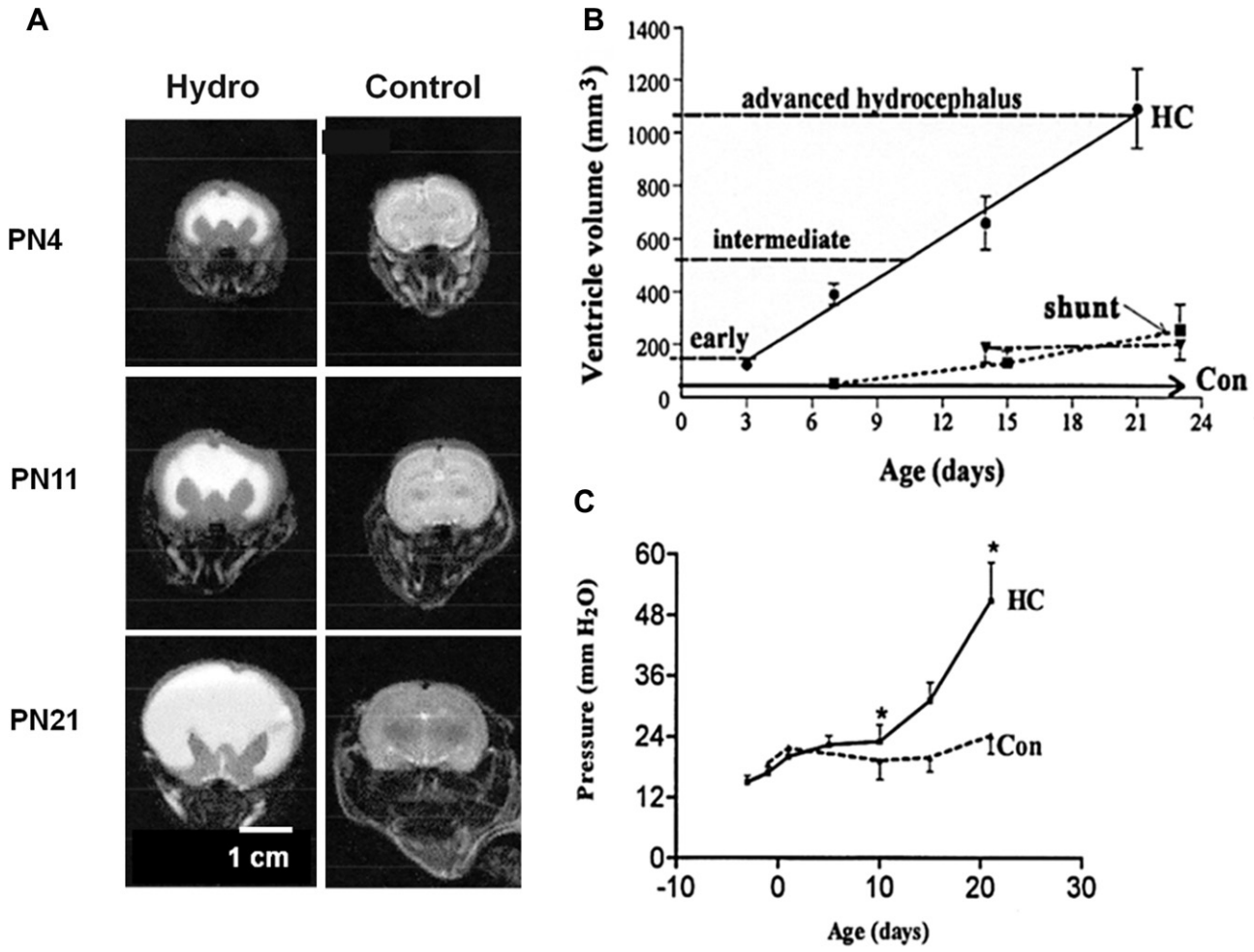

Fig. 1. Ventriculomegaly in the H-Tx rat. (A) T2-weighted magnetic resonance imaging (MRI) demonstrates the rapid progression of ventricular enlargement and tissue distortion at 4, 11 and 21 days of age. (B) Ventricular volumes from MRI studies illustrate the linear expansion of the cerebral ventricles in untreated hydrocephalic animals (HC) compared with non-hydrocephalic littermate controls (Con), as well as the cessation of ventriculomegaly in hydrocephalic animals shunted early (PN7) or late (PN14–15). (C) By contrast with the immediate expansion of the ventricles, intracranial pressure (ICP) does not begin to increase until PN10. Therefore, in this model there is a period, the beginning of which corresponds to late fetal development, when ventriculomegaly proceeds in the absence of elevated ICP. Modified from Jones et al.[55]

juvenile hydrocephalus arising from aqueductal stenosis. The advantage of congenital rat models is that ventriculomegaly occurs naturally, the brain is large enough for customized shunting, they are amenable to behavioral testing, cost is relatively low, and a wealth of data is available for baseline and pathological comparisons. Nevertheless, they are not ideal for long-term experiments unless shunting is performed (except if ventriculomegaly remains mild) and their size restricts the use of clinical shunt systems and pressure probes.

Several mouse models of hydrocephalus have provided important data on the pathogenesis of hydrocephalus.[19,24–32] The most widely used models are the SUMS/NP,[28,32] hy3,[31,33,34] transforming growth factor β1 overexpression,[30,35,36] hyh with a point mutation in α-SNAP [soluble NSF-attachment protein (NSF: N-ethylmaleimide-sensitive factor)] with ependymal denudation that precedes aqueductal stenosis,[37–40] fibroblast growth factor 2,[41] L1–cell adhesion molecule deficient,[27,42] aquaporin deficient,[43] hpy,[29] animals with a conserved forkhead/winged helix transcription factor gene,[29,44,45] heparin-binding epidermal growth factor (J.R. Madsen, unpublished observations), and collagen deficiencies.[46] McCarthy et al. have developed a double transgenic mouse model in which hydrocephalus can be induced through astrocyte activation (details below).[47,48] The obvious size limitations of mouse models limit the use of CSF shunts and invasive physiological sensors.

Time does not permit a thorough discussion of all the neonatal models of acquired hydrocephalus (reviewed by McAllister[16] and Johanson et al.[19]), but a few points should be made because much of our knowledge of the pathophysiology of hydrocephalus has been obtained in these models, primarily because they facilitate precise timing of experiments. Kaolin, an inert silicate, is the most common agent for inducing acquired hydrocephalus in newborn or infant mice, rats, rabbits, cats, dogs, and sheep. Injections of kaolin into the cisterna magna, fourth ventricle or cerebral aqueduct block the outflow of CSF from the ventricles to the subarachnoid spaces and thus induce obstructive hydrocephalus; kaolin injections have been made into the basal cisterns, and since the fourth ventricle outlets remain patent this technique produces communicating hydrocephalus. Although there have been no signs of direct pathological effects on structures distant to the injection site, some investigators still suspect that kaolin can produce a global intracranial inflammatory response, and thus possibly mask the effects of ventriculomegaly alone.

It is important to recognize that the presence of an expandable skull in congenital animal models significantly alters the biomechanical properties of the developing brain and certainly influences

Table 1
Summary of metabolic changes in untreated hydrocephalic H-Tx rats during the first 3 weeks of life.

Cortical metabolites	Age (days)		
	4	11	21
Membrane compounds			
Phosphomonoesters	*	*	*
Cholines, inositol			*
Energy catabolites			
ATP, P_i, phosphocreatine		*	*
Creatine			*
Amino acids and others			
Glutamate, glycine, aspartate, alanine			*
Taurine, *N*-acetyl aspartate			*

Data reproduced from Jones HC, Harris NG, Rocca JR, et al. *J Neurotrauma* 1997, vol. 14, p. 587.
*Statistically significant decreases.

the cellular pathology that follows. In adult animals, kaolin injections consistently produce a less severe ventriculomegaly. By contrast, kaolin injections in neonatal and juvenile animals usually cause rapidly progressing ventriculomegaly that reaches more severe proportions.

Finally, the importance of CSF shunting techniques[49–52] – now successfully applied to neonatal models – hold great promise for studies that attempt to determine the reversibility of pathological events.[53–55] Several caveats are worth noting, however: the ventricular catheters are usually custom-made and valves are seldom used, so caution is advised when comparing treatment outcomes using clinical hardware. Unfortunately, shunted animals, most notably the more fragile neonates, develop shunt malfunctions at approximately the same rate as human patients.

6. Progression of ventriculomegaly and the importance of intracranial pressure

In both experimental models and clinical scenarios, the assumption is almost always made that ventriculomegaly is proportional to an increase in intracranial pressure (ICP), and in many centers treatment decisions are based on ICP data. ICP increases do not occur always and occasionally negative pressures can be recorded, especially during postural changes. Jones et al.[55] have shown that the H-Tx rat model presents a good example of the caveats that should be considered when associating ventriculomegaly with ICP. By measuring ventricular volume with MRI and ICP in the same animals, these authors demonstrated clearly that ventriculomegaly progresses linearly and is not associated with raised ICP until the hydrocephalic animals are at least 10 days old (Fig. 1). Thus, the pathologies in H-Tx rats that are known to occur during the first week of life should not be attributed solely to raised ICP, but this pattern of delayed ICP increases could be a major factor underlying the poor response to shunt therapy in these animals when drainage is initiated late (12–15 days of age) rather than early (5–7 days of age). An appropriate clinical correlate of this situation occurs in premature infants with mild to moderate ventriculomegaly but no clear signs, i.e. a bulging anterior fontanel, of

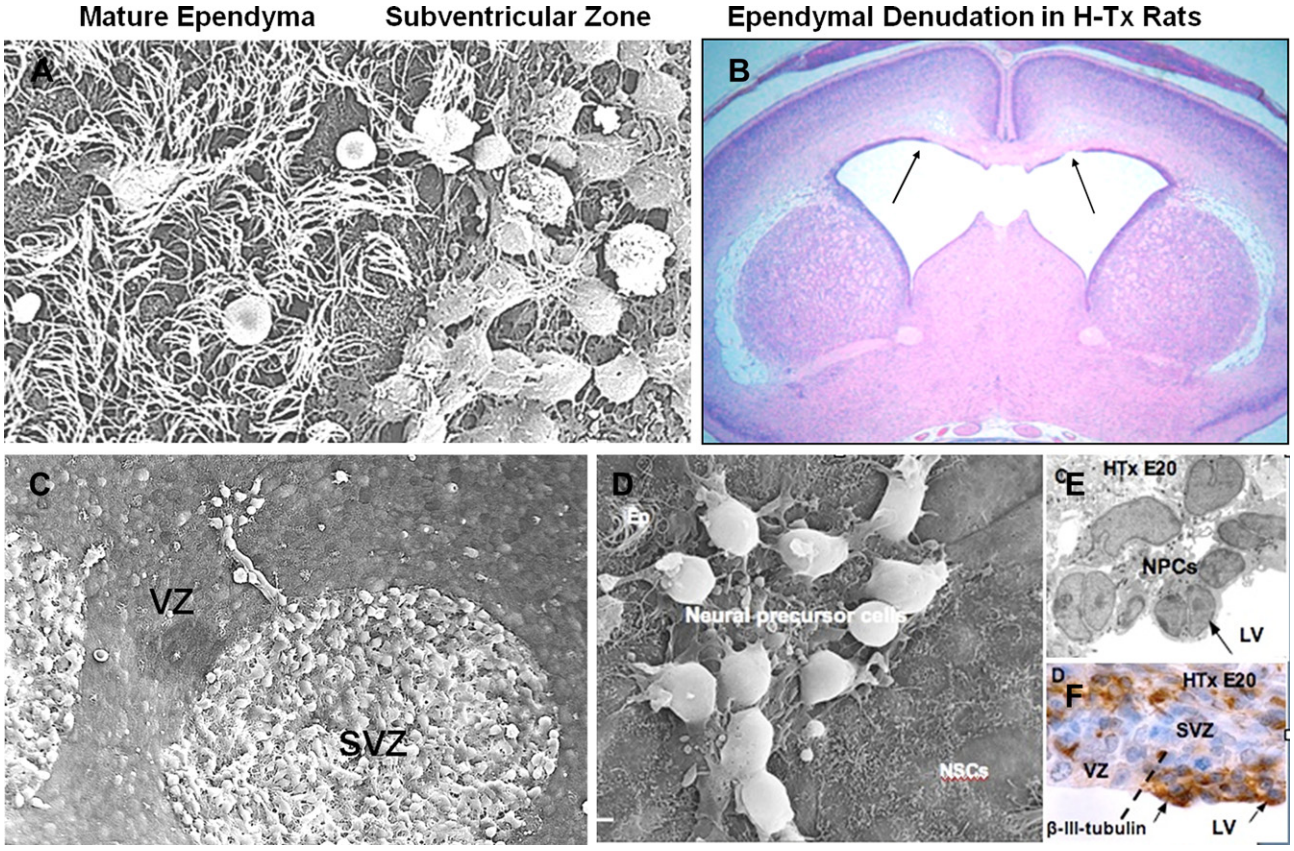

Fig. 2. Ependymal denudation. (A, B) Scanning electron micrograph (SEM) in (A) of the ventricular wall from a region similar to that shown in (B) from H-Tx rats at postnatal day 7; normal mature ciliated ependyma are adjacent to a region of denuded ependyma that exposes the subventricular zone. (C) Another SEM showing the patchy pattern of exposed subventricular zones (SVZ). (D) SEM at high magnification showing exposed neural precursors. (E, F) The identity of these exposed cells as embryonic day 20 neural progenitors is confirmed with transmission electron microscopy (E) and immunostaining for β-III-tubulin (F). LV, lateral ventricle; NSCs, neural stem cells; NPCs, neural progenitor cells; VZ, ventricular zone. Illustrations courtesy of Dr Esteban Rodriguez.

high ICP. In these cases, clinical management is usually very conservative with shunt surgery delayed as long as possible until the infant has matured and surgical risk has been reduced. Experimental results, however, suggest that brain damage can occur during the waiting period.

7. Metabolic deficits begin early

Rodent models have also demonstrated that metabolic impairments occur quite early in the progression of ventriculomegaly. Magnetic resonance spectroscopy studies[55] on the cerebral cortex of the H-Tx rat have shown that significant decreases in membrane compounds occur as early as 4 days of age (compared with the relatively mild ventriculomegaly shown in Fig. 1) and progress sequentially to involve energy metabolites and finally amino acids, proteins and neurotransmitters (Table 1). At 4 days of age, these animals show no signs of behavioral or neurological deficits, yet profound metabolic deficits are clearly present. Most importantly, shunting these animals at 4–5 days of age restores metabolite levels to normal whereas shunting at 10 days of age fails to reverse the deficits.[56]

8. Altered neurogenesis and the role of trophic factors in the CSF

For many years the over-riding assumption was that all of the cytopathology identified during neonatal hydrocephalus was caused only by the expanding ventricles and raised ICP with little regard for the potential proteins, electrolytes and other substances that inhabit the CSF. Fortunately, in 1998, Miyan et al. designed an elegant experiment that clearly showed the impact of altered CSF in hydrocephalus.[57] They added three types of CSF taken from the lateral ventricles of (a) normal Sprague–Dawley rats, (b) non-hydrocephalic H-Tx rats, and (c) phenotypically hydrocephalic H-Tx rats to cultured neurons. A 10-fold reduction in proliferation and an accumulation of cells in S-phase occurred only in the cultures receiving 'hydrocephalic' CSF. This was the first indication that neurotrophic factors could be present in the CSF of hydrocephalic brains and that these trophic factors could impair neurogenesis. An important follow-up study[58] identified impairments in folate metabolism as the most likely mechanism responsible for cell-cycle arrest in neural progenitors; in particular, low levels of 10-formyltetrahydrofolate dehydrogenase in CSF were associated with ventriculomegaly in H-Tx rats.

9. Ependymal denudation and its influence on neurogenesis

Another important mechanism that could be genetically driven or arise as a consequence of compression and stretch on the walls of the ventricles now appears to play a major role in the pathophysiology of congenital hydrocephalus. Since their original finding nearly 10 years ago, Rodriguez et al. have continued to reveal the impact of ependymal denudation in the pathogenesis of hydrocephalus.[59–63] At the cytoarchitectural level, this phenomenon is simply a complete loss of the ependymal cells such that bare patches appear through which the underlying periventricular white matter and subependymal region can be seen (Fig. 2). Scanning electron microscopy illustrates beautifully the loss of mature ciliated ependymal cells exposing cells of the subventricular zone to ventricular CSF. Transmission electron microscopy and immunostaining for β-III-tubulin have confirmed that the exposed cells are in fact neural progenitors.

These ependymal changes occur in fetal mice as well as rats, and painstaking studies of the hyh mouse model of congenital hydrocephalus have shown that ependymal denudation begins in the midbrain as early as embryonic day 12.5 (E12.5) and progresses rostrally until the entire lateral ventricle exhibits patches of ependymal cell loss on E15.5 (Fig. 3). The timing of this 'wave' of denudation is critical. It is not until E15.5 that hyh mice first begin to show evidence of ventriculomegaly; thus, it appears that ependymal denudation plays a role in *causing* hydrocephalus.

To help understand how ependymal denudation may contribute to the cause of congenital hydrocephalus, it is important to recall some basic principles of embryonic neurodevelopment. All cells of the developing mammalian brain are born in two germinal zones associated with the ventricular walls, the ventricular zone (VZ) or ependymal layer and the subventricular zone (SVZ) (Fig. 4).[64–66] The VZ is a pseudostratified neuroepithelium that contains multipotent radial glia/stem cells, often designated as neural stem cells (NSCs). A population of NSCs differentiates into immature ependyma, which, during the first postnatal week in mice, mature into ependyma. In humans, ependymal cell differentiation starts at about the fourth week of gestation and is completed around the 22nd gestational week. The SVZ is located underneath the VZ along

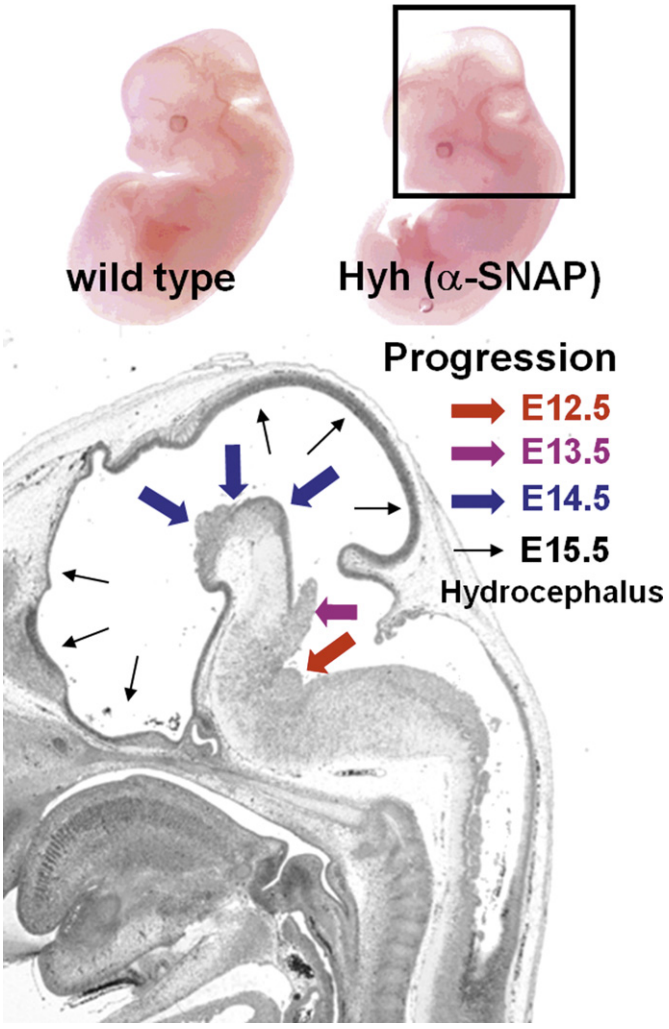

Fig. 3. Progression of ependymal denudation in the Hyh mouse with congenital hydrocephalus. In the histological section taken from the region within the square, the 'wave' of denudation has been illustrated beginning at embryonic day 12.5 (E12.5) in the midbrain and progressing in a rostral direction through the third and lateral ventricles. By E15.5, when ventriculomegaly first appears, denudation has reached all parts of the lateral ventricles. α-SNAP, soluble NSF-attachment protein (NSF: N-ethylmaleimide-sensitive factor). Courtesy of Dr Esteban Rodriguez.

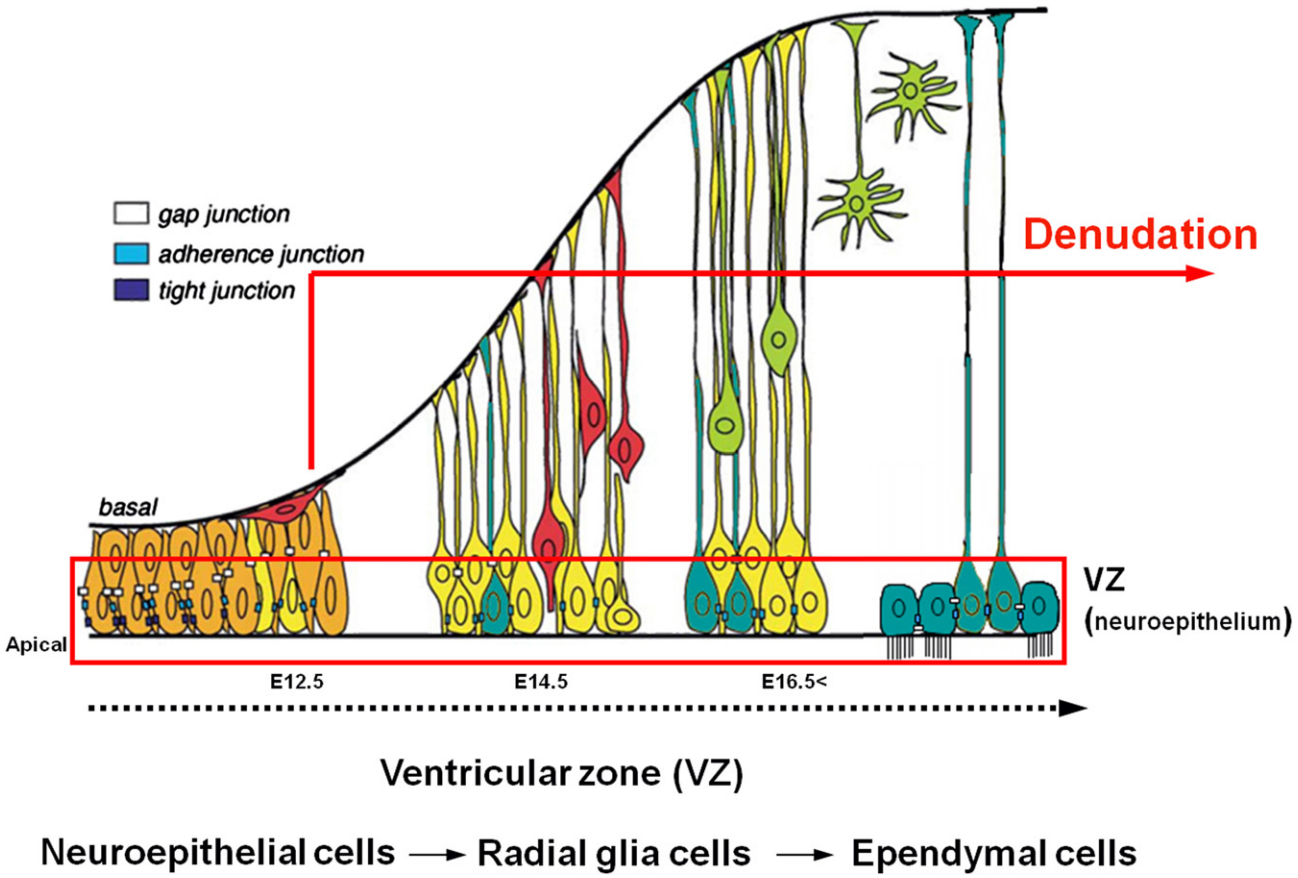

Fig. 4. Schema of the stage in gestation when the neuroepithelium first becomes affected by ependymal denudation. Note that exposure of the neural progenitor cells occurs from embryonic day 12.5 (E12.5) onward when neurogenesis has been initiated.

the walls of the lateral ventricles of the embryonic brain; it contains the neural precursors, which lose contact with the ventricular surface.

Radial glia, immature ependyma and mature ependyma all have distinct phenotypes and certainly play quite different roles. However, they all rely on junctional proteins during development. Up to E12, neuroepithelial cells lining the neural tube are joined together by gap, adherens and tight junctions. From E12 on, tight junctions are lost and cell-to-cell adhesion only relies on gap and adherent junctions. It is exactly at this time, E12, when disruption of the neuroepithelium starts in the mutant hyh mouse (Fig. 3). A critical finding by Ferland et al.[59] helps explain the cellular mechanism underlying ependymal denudation: hyh mutants contain mutated genes encoding for proteins involved in intracellular trafficking and transport of junction proteins to the plasma membrane. Thus, disruption of the ependyma of the VZ probably arises from a final common pathway involving alterations of vesicle trafficking, abnormal cell junctions and loss of neuroependymal integrity. How VZ disruption affects the SVZ is still not clear, and conflicting opinions exist on whether exposure to CSF is beneficial[67,68] or harmful.[57]

Initially, skeptics of the role that ependymal denudation might play in the pathogenesis of congenital hydrocephalus were convinced that this was a phenomenon that occurred only in rodents. However, Sival et al.[69,70] and Jimenez et al.[63] have demonstrated clearly that ependymal denudation occurs in humans with hydrocephalus. For example, in a careful immunocytochemical study of five fetuses with spina bifida aperta and hydrocephalus compared to six normal fetuses, all of gestational age 37–40 weeks, Sival et al.[69] reported that ependymal denudation was absent in controls, but 'imminent ependymal denudation (with abnormal subcellular location of junction proteins)' and 'protrusion of neuropile' into the ventricle was evident in the hydrocephalic fetuses. Based on the similarities between their clinical cases and the alterations observed in H-Tx rats and hyh mice, the authors suggested that 'abnormalities in the formation of gap and adherent junctions result in defective ependymal coupling… and ependymal denudation, leading to hydrocephalus.'.

10. Influence of ependymal denudation and the subcommissural organ aqueductal stenosis

Aqueduct stenosis is a key event in the development of many forms of congenital hydrocephalus although the causes are not well known. In 1954 Overholser et al.[71] found that offspring littered by rats maintained on a diet deficient in folic acid and/or vitamin B_{12} lack a subcommissural organ (SCO) and develop hydrocephalus, and hypothesized that SCO dysfunction leads to aqueductal stenosis and congenital hydrocephalus. The SCO is a brain gland that develops early in ontogeny.[40,72] It secretes negatively charged sialoglycoproteins into the CSF, where they either aggregate to form Reissner fiber (RF) or remain CSF-soluble.[40] The two main secretory products are SCO-spondin and transthyretin.[40,73] SCO-spondin promotes neuronal growth and differentiation. SCO-spondin-related compounds remain soluble in the fetal and adult CSF and follow the CSF flow. By the permanent addition of new molecules to its rostral end, RF continuously grows and extends along the cerebral aqueduct, fourth ventricle, and central canal of the spinal

Fig. 5. Immunostained sections of the rostral portion of the cerebral aqueduct in H-Tx rats illustrating the ependymal alterations that lead to aqueductal stenosis and hydrocephalus. (A) Non-hydrocephalic animals at E18 exhibit a complete subcommissural organ (SCO, visualized with an antibody to SCO-spondin) that produces a continuous Reissner's fiber (RF) that extends through the cerebral aqueduct. (B, C) At E18 the middle portion of the SCO is absent and a Reissner's fiber has not formed; just one day later the juxtaposed walls of the cerebral aqueduct without an SCO have fused and blocked the pathway for CSF absorption. Courtesy of Dr Esteban Rodriguez.

cord. Immunological blockage of SCO secretions and several mutants with abnormal SCO all develop aqueduct malformations and hydrocephalus, largely substantiating the early hypothesis of Overholser.[71] A good body of evidence, mostly from Dr Rodriguez, indicates that RF-glycoproteins are essential for a normal flow of CSF throughout the cerebral aqueduct, and that the absence of RF could cause aqueduct obliteration or a turbulent CSF flow through CA, which, in turn, could lead to hydrocephalus.[40]

Ependymal denudation and disruption also appear to play a major role in one of the classical features of congenital hydrocephalus, aqueductal stenosis. Like the ependymal changes noted in the lateral ventricles, the initial observations on the cerebral aqueduct were made on hyh mice and H-Tx rats and involve incomplete formation of the SCO. In these animals the SCO is formed by three distinct zones whose differentiation should be controlled by different genes (Fig. 5). In the H-Tx rat, the mutation results in the lack of differentiation of the middle third of the SCO and in the absence of Reissner's fiber (RF). This malformation expresses early in development and precedes the stenosis/obliteration of cerebra aqueduct occurring at E19. The malformation of the middle third of the SCO is the primary cause of aqueduct obliteration and onset of hydrocephalus.

Recent clinical studies have corroborated the fact that ependymal denudation occurs in the cerebral aqueduct in patients with spina bifida aperta and hydrocephalus or Chiari II malformation.[69] Denudation is evident in the aqueduct (and lateral ventricles) as early as 16 weeks of gestation and with time the denuded area becomes infiltrated with reactive astrocytes.

As mentioned at the outset, whereas it is clear that ependymal denudation and SCO impairment play important roles in the pathogenesis of aqueductal stenosis, a recent study in our laboratory has shown that SCO secretions can be reduced by ventriculomegaly alone. In neonatal felines with kaolin-induced obstructive hydrocephalus, the levels of SCO-spondin in ventricular CSF samples gradually decrease over a 14-week period (unpublished findings). Initially the levels of SCO-spondin are normal even though ventriculomegaly is severe, but after about 3 weeks of persistent hydrocephalus the levels begin to decline appreciably. This important finding suggests that SCO impairments can be a consequence of ventriculomegaly as well as pathogenic.

11. Role of astrocytes in the development of hydrocephalus

In hydrocephalus, gliosis is known to occur, but the time course and permanence of the reaction are still not clear.[12,13,15,74–79] It has been suggested by many investigators,[15] including ourselves,[74] that glial scar formation is a permanent fixture in hydrocephalic brains, even those that have been shunted successfully. Our previous studies have shown that GFAP (glial fibrillary acidic protein, specific for astrocytes) RNA levels increase with the progression of hydrocephalus in H-Tx rats[21,52] and following kaolin injections in neonatal rats[80] and kittens.[81] Additionally, Mangano et al. showed that microglial cell proliferation and activation increased in regions of the sensorimotor cortex and auditory cortex during the progression of hydrocephalus in moderately affected H-Tx rats.[74] Clinically, increased levels of GFAP protein have been found in the CSF of patients with normal pressure hydrocephalus, and patients who developed secondary hydrocephalus due to subarachnoid hemorrhage,[82] and the possibility of using GFAP protein levels as a diagnostic tool for hydrocephalus is currently being explored.[83] Finally, experimental studies on both congenital rodent models and acquired hydrocephalus have demonstrated that shunting can reduce the amount of GFAP protein and RNA present in the cerebral cortex.[52,81]

It is likely that gliosis may contribute to the altered mechanical properties believed to occur in hydrocephalic brains[84] so that they become more rigid (less compliant). The importance of these properties in hydrocephalus is illustrated by the finding that reduced compliance, measured using the pressure–volume index, serves as one of the best predictors of shunt success rather than measurements of ventricular size.[85] Furthermore, compliance and CSF outflow resistance has a direct affect on the fundamental parameters that regulate shunt function. Therefore, progress in shunt design has been hampered by the lack of information about the relationship between the resistance to CSF flow and scar formation.

With the development of a novel double-transgenic model of hydrocephalus by McCarthy et al.,[47,48] the role of astrocyte activation as a causative factor in hydrocephalus has been identified. These investigators cleverly manipulated a mouse line expressing the Gi-coupled Ro1 receptor activated solely by synthetic ligands (RASSL) in astrocytes. Crossing the Ro1 line (under the tetO promoter) with a tet-transactivator (tTA) line under control of a fragment of the human glial fibrillary acidic protein promoter allowed expression of Ro1 in astrocytes only. Most importantly, astrocyte activation could be regulated using doxycycline (dox) to bind tTA, preventing it from binding the tetO promoter, thus inducing hydrocephalus at any age. When astrocytes were activated during gestation or the first month of life, these animals developed hydrocephalus similar to that found in H-Tx rats or hyh mice investigations. Ependymal denudation was present, but it appeared subsequent to severe ventriculomegaly, suggesting that it is a secondary effect rather than a cause. Furthermore, aqueductal stenosis was not manifest at any time nor were any obstructions observed within the ventricles; thus this model appears to be one of communicating hydrocephalus.

12. Role of neuroinflammation in the pathophysiology of congenital hydrocephalus and the potential for therapeutic interventions

To expand on the reactive astrocytosis known to occur during hydrocephalus, it now appears that neuroinflammation is a consistent consequence of ventriculomegaly and a major component of the pathophysiology of hydrocephalus. Further work in our laboratory has shown that microglia react much like astrocytes in both congenital[52] and acquired neonatal hydrocephalus.[80] For example, in rats receiving intracisternal kaolin injections on postnatal day 1, GFAP and Iba-1 (ionized calcium binding adapter molecule 1 to detect microglia) proteins were significantly elevated at postnatal day 21 and were associated with both moderate and severe degrees of ventriculomegaly. A microarray analysis of 33,951 genes identified significant (1.5-fold) changes in 1729 genes. Many of these genes were prominently associated with cytokine and antigen-presenting pathways, indicating that an inflammatory response accompanies progressive ventriculomegaly.

Minocycline, a derivative of the well-known antibiotic tetracycline, has recently shown promise as a specific inhibitor of microglial cells, one of the main elements of glial scar formation in hydrocephalus. Its promise as a neuroprotective agent is illustrated by the recent initiation of clinical trials in Parkinson disease.[86] Since its initial demonstration as an anti-inflammatory agent,[87] minocycline has been shown to have multiple benefits in brain injury.[88,89] Recently, we administered minocycline intraperitoneally to H-Tx mice after they had developed severe hydrocephalus, at postnatal days 15-21 just prior to killing. We reasoned that this period would correspond to the approximate time that a neonate or infant would present to a clinic with signs and symptoms of hydrocephalus. Minocycline treatment significantly reduced the number of microglial cells in the cerebral cortex of hydrocephalic animals by 71%. Likewise, minocycline treatments produced a cortical mantle that was thicker by as much as 58% in the parietal cortex and 180% in the occipital cortex, which is usually thinned more than other cortical regions. Finally, astrocytes, which are not expected to be influenced directly by minocycline, also exhibited increases after minocycline treatment. These encouraging results lend support to the idea that neuroinflammation could be modulated by pharmacologic treatments. These interventions, which most likely would be supplemental to the placement of CSF shunts, could be performed only during periods of tissue vulnerability, such as during shunt malfunctions or in premature neonates awaiting surgery.

Practice points

- Ependymal denudation has been observed recently in spina bifida aperta and hydrocephalus.
- Ependymal denudation may contribute to the pathogenesis of human hydrocephalus.
- The pathophysiology of congenital hydrocephalus is multifactorial and complicated.

Research directions

- Rodent models of congenital hydrocephalus provide valuable data.
- Potential pharmacological interventions are promising and should be explored.
- Ependymal denudation and subcommissural organ impairments play a major role in the pathogenesis of congenital hydrocephalus.
- Neuroinflammation plays a major role in the pathophysiology of hydrocephalus and pharmacological modulation should be developed for supplemental treatment.

Conflict of interest statement

None declared.

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