Animal Models of Hydrocephalus

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Abstract

Hydrocephalus is a neurological condition characterized by altered cerebrospinal fluid (CSF) flow leading to an accumulation of CSF inside the cranial vault. Neuropathogenesis associated with hydrocephalus has been elucidated by pathological studies of human brains and through experimental and genetic animal models. Experimental animal models have been developed in numerous species using a variety of methods and agents to induce hydrocephalus or through genetic mutations in rodents. Each of these animal models has been described briefly in this review, along with the basic strengths and weaknesses of each model. Although none of these models can fully mimic the human condition, they each provide fundamental knowledge contributing to understanding more about the pathogenesis of hydrocephalus and its underlying causes.

Keywords

Hydrocephalus, Animal Models, Kaolin, Mechanical, Genetic

1. Introduction

The pathogenesis of brain damage in hydrocephalus has been elucidated by pathological studies of human brains and through the use of experimental animal models. Experimental animal models have been developed in a range of species using a variety of methods to induce hydrocephalus or through genetic mutations in rodents. These models have been assessed in different reviews [1]-[6]. The following discussion will briefly describe each of the experimental models, along with the basic advantages and disadvantages of each model. Although none of these models can fully mimic the human condition, they each provide important contributions of understanding more about the pathogenesis of hydrocephalus and how to potentially treat it effectively.
2. Kaolin Injection

The most common method of inducing experimental hydrocephalus is the intracisternal kaolin injection model [1]. This model was introduced in the 1930s [7] [8]. A dose of 0.01 - 0.2 mL of 20% - 25% suspension of kaolin clay (aluminum silicate) is injected into the cisterna magna either through surgical exposure of the cistern and brainstem or by inserting a needle tip percutaneously. Kaolin deposited at the base of the fourth ventricle spreads in the subarachnoid space, where it induces an inflammatory reaction and fibrous scarring in the meninges. This resembles the scarring that develops following meningitis or hemorrhage and leads to an obstruction of the CSF pathways close to the fourth ventricle apertures and ventricular enlargement ensues [4] [5] [7] [9]. The species that have been examined include mice [10] [11], rats [12] [13] [14] [15] [16], guinea pigs [17] [18] [19], rabbits [20], cats [8] [21] [22] [23], ferrets [24] [25], dogs [9] [26] [27] [28], pigs [29], sheep [30] [31] [32], and monkeys [30] [32]. The main disadvantage of kaolin induction is that the inflammatory reaction, composed of macrophages and CD4- and CD8-positive lymphocytes [33], might confound interpretation of microglial reactions in these animal models. Second, if performing the percutaneous needle injection method, it is possible to cause damage by accidental punctures into brainstem structures, particularly in neonatal animals. Third, although the dose and concentration of kaolin injected likely play a role in the rate of ventriculomegaly and pathology, age of the animal and mode of inducing hydrocephalus, whether using kaolin or otherwise, are important factors in hydrocephalic pathological outcomes [3] [24] [34]. Even when using the same dose and concentration, there is variable dispersion of kaolin in the subarachnoid space, and this may account for the relatively unpredictable rate and magnitude of ventricular dilatation that transpires. However, these are outweighed by the practical matter that the kaolin model is a simple, inexpensive, and consistent way of inducing hydrocephalus in experimental animals [5] [34].

3. Silicone Oil Injection

Another method of inducing hydrocephalus in animal models is through an intracisternal injection of viscous silicone oil. This creates a purely mechanical obstruction for the outflow of CSF from the fourth ventricle to the subarachnoid space [20] [35]. The method has also been successfully used in a few animal species including rats [36], rabbits [37] [38] [39] [40] [41], and dogs [35] [42] [43]. The silicone oil used in this model is apparently an inert substance, so it does not produce the same inflammation of the meninges and scarring that kaolin induces [35] [41] [42]. It should be noted though that silicone oils do lead to some intraocular inflammatory response when used in the treatment of retinal detachment [44], but it is not certain how this translates to the brain. Like kaolin, the silicone oil model is easy and inexpensive to perform [1] [5]; however, it does not produce severe ventricular expansion or sustained elevated intracranial
pressure [1] [5] [39], and thus, it is not an effective model of chronic infantile hydrocephalus. Some studies used a silastic elastomer solution that hardens quickly to improve the rate of ventriculomegaly attained [45] [46].

4. Mechanical Obstructions and Toxins

Over the 20th and 21st centuries, various other substances/agents have been used to induce hydrocephalus by implanting a mechanical plug that leads to hydrocephalus. Some of these substances include cotton or cellophane cylinders implanted in the cerebral aqueduct of adult dogs [9] [47] [48]; India ink [49], small pieces of laminalia [6], and cyanoacrylate glue [50] [51] among others. These substances will typically cause an obstructive form of hydrocephalus either by blocking the fourth ventricle and/or access to the subarachnoid space through the apertures or by inducing aqueductal stenosis, which will in turn lead to ventriculomegaly. Cotton and cellophane plugs have successfully induced hydrocephalus in large animals, but they involve an invasive, major surgical procedure that likely damages brainstem structures, so they should only be limited to acute experiments [5]. Cyanoacrylate glue also induces obstructive hydrocephalus in the fourth ventricle, where it quickly cures, and this potentially prevents CSF leakage by adhering to the ependymal and pial layers of the brainstem and cerebellum without distorting surrounding brain tissues [5]. It has been shown to work in large animals (i.e., dogs) with gyrencephalic brains, like humans, but it is also expensive and involves a complicated technical procedure that has not always worked in other animal models [1] [5] [52]. Another issue is that these mechanical obstructions limit the experiments to have an acute nature, and thus may only represent trauma-induced clinical hydrocephalus in humans but not progressive forms of the condition. An additional implantation method is balloon insertion in the ventricles of lambs [53] [54] [55]. Unlike the other techniques described above, the balloon implantation method induces a form of communicating or non-obstructive hydrocephalus because there is no point of obstruction and does not change the mean CSF pressure [53] [54] [55]. However, it elevates the pulse wave of the CSF in the ventricles [56], which could have other implications.

Different toxins have been administered or fed to pregnant rats, which successfully produced hydrocephalic offspring. Some of these substances include trypan blue [57] and tellurium [58] [59] [60]. These substances will lead to obstructive hydrocephalus by blocking the cerebral aqueduct or by closure of the subarachnoid space, and ventricular enlargement will follow in these rats. Unfortunately, these toxins likely cause brain damage or alter normal developmental sequences to induce congenital defects that subsequently lead to ventriculomegaly, and often death will occur by the end of the second week of life [59] [61].

5. Molecular Fibrosis Manipulation

Molecular agents associated with the fibrotic pathway have also been injected or
overexpressed in animal models to induce obstructive hydrocephalus. Basic fibroblast growth factor (FGF-2) [62] [63] has been injected/implanted in the cisterna magna of rats, rabbit, dogs, and/or marmosets. Intrathecal injection or transgenic overexpression of transforming growth factor-beta 1 (TGF-β1) has also successfully induced hydrocephalus in mice [64] [65] [66] [67] [68]. Basic FGF-2 and TGF-β1 injections are relatively easy procedures that both presumably create a fibrotic obstruction in the subarachnoid space [1] [5] [66] [69], but they are expensive procedures that might have a direct impact on different brain cells, including synapse formation and neuronal migration [1] [70] [71].

6. Blood and Blood-Related Injections

More recently, several studies have established rodent models of post-hemorrhagic hydrocephalus through intraventricular injection of blood directly into the lateral ventricles of rats [72] [73] [74]. Other studies have successfully performed the same intraventricular injection(s) with substances found in blood serum and/or plasma either alone or in conjunction with blood itself, including thrombin [75] [76] and FeCl₃ [77] [78] in rats, along with lysophosphatidic acid (LPA) in mice [79]. All of these studies have also examined non-surgical therapeutic agents to treat the experimental hydrocephalus with varied efficacy, which sheds more light on the neuropathology associated with hydrocephalus along with the potential neuroprotective effects of various pharmacological treatments. In addition, some of these models, such as the LPA model in mice [79], resemble different human fetal forms of the hydrocephalus caused by hemorrhage [80] [81]. However, blood-brain-barrier disruption could occur using these models [75], and hemorrhage is only one of the many causes of hydrocephalus. In addition, it is important to examine these blood injection models further without therapeutic intervention to learn more about the neuropathology associated with chronic hydrocephalus.

7. Genetic Models

In humans, hydrocephalus manifests due to a variety of causes and can occur across the lifespan or be present at birth. Congenital hydrocephalus can be linked to genetic causes, yet until recently, there were only a few genes linked to the condition, including the X-linked L1 cell adhesion molecule (L1CAM) [82]. This recent study has shown that there may be increased genetic heterogeneity associated with hydrocephalus. There are also genetic models of hydrocephalus that have been discovered and studied over the last century, but very few are linked to the same genes as humans. The genetic models include the hydrocephalus Texas (H-Tx) rat, the LEW/Jms rat, the L1CAM mutant mouse model, the hydrocephalus-3 (hy-3) mouse, the hydrocephalus with hop gait (hyh) mouse, the SUMS/NP mouse, the hpy mouse, and a double transgenic mouse model, among others. The hydrocephalic H-Tx rat is a spontaneous neonatal hydrocephalus model that develops aqueductal stenosis and ventricular
enlargement beginning at approximately 18 days gestation [83] [84], while the LEW/Jms rat strain came from an inbred strain of Wistar-Lewis rats that exhibit a similar onset and pathophysiology to the H-Tx rat [85] [86]. The H-Tx rat has been studied extensively and displays adverse effects in the germinal epithelium (GE) including reduced cell proliferation, impaired cell cycling, and/or increased cell death and arrested migration of glial cells from the GE into the cerebrum, which are hypothesized to be associated with impaired signalling molecules carried by the CSF [87]-[93]. Its phenotypic characterization is predictable, and surgical intervention can treat this inherited form of hydrocephalus [5] [94]. Additionally, research is uncovering the potential roles that folate imbalance plays in the defects associated with the early-onset of hydrocephalus, where maternal administration of folic acid increases the incidence of hydrocephalus, while combined folinic acid and tetrahydrofolate decrease the incidence [95]. Despite these breakthroughs, the underlying genetic cause of this partially penetrant disorder is still unknown [96] [97] [98], which raises issues about its applicability. There is also concern that there are potential brain abnormalities in nonhydrocephalic “normal” H-Tx rats because they perform worse than Sprague-Dawley rats on behavioural tests [99]. It is also expensive to maintain the breeding colony for these rats [1] [5].

As indicated, there are also spontaneous mutant mice that exhibit a hydrocephalic phenotype. The L1 CAM mutant mouse often displays ventricular expansion, cerebral cortex pyramidal neuron defects, and shrunken hippocampus, corticospinal tract, and corpus callosum [100] [101]. Meanwhile, the hy-3 mouse was initially discovered in the 1940s and was suspected to be the result of a pleiotropic gene [102]. Research in this model continued in the 1960s and 1970s, where the inheritance and pathogenesis were investigated, which revealed impairment to the choroid plexus and ependymal layer [103] [104]. However, it was not until 2003 that the discovery of the autosomal-recessive frameshift mutation in the Hydin gene caused this lethal form of perinatal onset communicating hydrocephalus [105], which may manifest due to impairment of ependymal ciliary motility [106]. With further understanding of the genetic cause of hy-3, it is becoming more feasible to investigate this model further. However, like the H-Tx rat, it is expensive to maintain the breeding colony [1] [5]. Another mutant model is the hyh hydrocephalic mouse that is associated with a domed head, appreciably reduced cerebral cortex, and a lack of communication between the caudal aqueduct and fourth ventricle, which subsequently leads to ventricular enlargement that is typically lethal between a few weeks to 2 months of age [107]. However, the hyh mouse is a complex genetic model involving a hypomorphic missense mutation of the soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein alpha-S-nitroso-N-acetylpenicillamine (Napa α-SNAP) gene mapped to the proximal end of chromosome 7, which is associated with mRNA instability [107] [108] [109]. This is believed to disturb neural cell determination by disrupting cortical progenitor pools and laminar organization, as well as the localization of several apical proteins implicated in regulating neural
cell fate. It has other effects on cell processes including disorganization and reduction of both proliferative and neural progenitor cells (NPCs) in the subventricular zone (SVZ) [110], but the cause of these effects is not clearly understood. It also displays the hop gait phenotype, which is not necessarily associated with hydrocephalus. In addition, mutant mouse models are too small for surgical interventions, and thus, there are concerns about their applicability to the human situation [5].

The SUMS/NP mouse is an inbred strain involving a recessive gene that is likely autosomal and develops congenital hydrocephalus with ventricle enlargement around E16 but is explicitly visible by P3-P4 in about 13% of matings between heterozygous parents [111] [112]. These animals develop progressively severe hydrocephalus with expanded lateral and third ventricles and reduced or absent cerebral aqueduct, and they die shortly after weaning. The hydrocephalus associated with polydactyly (hpy) mice involves a recessive mutation on chromosome 6 where homozygous mice exhibit a hopping gait, male sterility, scoliosis, and develop non-obstructive hydrocephalus postnatally around P6 and most die by P14 [113] [114] [115]. The hpy mice were also originally observed as offspring of X-irradiated mice [114] [116], but the specific factors associated with hydrocephalus development are unknown [117]. The unique double transgenic mouse model of communicating hydrocephalus shows severe ventricular enlargement and ependymal denudation and can be induced at any age because of doxycycline, which binds to tet-transactivator (tTA) and regulates astrocyte activation [118] [119]. It involves crossing of the G1-coupled Ro1 receptor activated solely by synthetic ligands (RASSL) in astrocytes mouse line with a tTA mouse line that has a fragment of human glial fibrillary acidic protein (GFAP) promoter that enables expression of Ro1 in astrocytes only. Despite the benefits of these mouse models in understanding the neuropathology of hydrocephalus, these models are limited because of the difficulties in incorporating surgical treatment, such as ventricular shunts, primarily due to the small size [120].

8. Summary

Researchers have unveiled different spontaneous mutant models of hydrocephalus, while others have induced experimental hydrocephalus using numerous agents with some successfully working in different animal models. All of these studies have imparted important information to understand the causes of and potential treatments for hydrocephalus. Because of them, much has been discovered about the neuropathology of this condition. However, the above discussion has made it evident that none of them are perfect in mimicking the human condition, for which there is still no definitive cure.

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**Abbreviations**

CSF—cerebrospinal fluid
FGF—fibroblast growth factor
GE—germinal epithelium
GFAP—glial fibrillary acidic protein
H-Tx—hydrocephalus Texas
hpv—hydrocephalus associated with polydactyly
hy-3—hydrocephalus-3
byh—hydrocephalus with hop gait
L1 CAM—L1 cell adhesion molecule
LPA—lysophosphatidic acid
Napa α-SNAP—N-ethylmaleimide-sensitive factor (NSF) attachment protein alpha-S-nitroso-N-acetylpenicillamine
NPCs—neuronal progenitors cells
RASSL—receptor activated solely by synthetic ligands
SVZ—subventricular zone
TGF-β1—transforming growth factor-beta 1
tTA—tet-transactivator