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Brain damage in neonatal rats following kaolin induction of hydrocephalus

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Abstract

Neonatal and congenital hydrocephalus are common problems in humans. Hydrocephalus was induced in 1-day-old rats by injection of kaolin into the cisterna magna. At 7 and 21 days, magnetic resonance (MR) imaging was used to assess ventricle size, then brains were subjected to histopathological and biochemical analyses. Hydrocephalic pups did not exhibit delays in righting or negative geotaxis reflexes during the first week. At 7 days, there was variable ventricular enlargement with periventricular white matter edema, axon damage, reactive astrogliosis, and accumulation of macrophages in severe but not mild hydrocephalus. Cellular proliferation in the subependymal zone was significantly reduced. The cortical subplate neuron layer was disrupted. In rats allowed to survive to 21 days, weight was significantly lower in severely hydrocephalic rats. They also exhibited impaired memory in the Morris water maze test. Despite abnormal posture, there was minimal quantitative impairment of walking ability on a rotating cylinder. At 21 days, histological studies showed reduced corpus callosum thickness, fewer mature oligodendrocytes, damaged axons, and astroglial/microglial reaction. Reduced myelin basic protein, increased glial fibrillary acidic protein, and stable synaptophysin content were demonstrated by immunochemical methods. In conclusion, impairment in cognition and motor skills corresponds to ventricular enlargement and white matter destruction. Quantitative measures of weight, memory, ventricle size, and myelin, and glial proteins in this neonatal model of hydrocephalus will be useful tools for assessment of experimental therapeutic interventions.
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Keywords: Axon; White matter injury; Cerebral ventricle; Myelin; Oligodendrocyte; Subventricular zone

Introduction

Hydrocephalus is a common neurological condition of childhood characterized by dilation of the cerebral ventricles, usually caused by obstruction of cerebrospinal fluid (CSF) flow. Axon damage in the periventricular white matter is one of the earliest pathological consequences of ventricular dilation in humans and animals (Del Bigio, 1993) and can account for many of the behavioral deficits (Del Bigio et al., 2003). The pathophysiology of hydrocephalus-induced brain damage is multifactorial, with contributions made by gradual physical stretching and compression of brain, ischemia with calcium-mediated axoskeletal damage, and possible accumulation of metabolic waste products (Del Bigio, 1993, 2004; McAllister and Chovan, 1998). Infantile hydrocephalus results from congenital brain malformations, intraventricular hemorrhage (IVH), and meningitis. The former can be modeled with H-Tx rats (Jones and Bucknall, 1988) or mutant mice (Bruni et al., 1988). Post-IVH hydrocephalus can be created by double intraventricular blood injection in 7-day-old rats (Cherian et al., 2003), although in our experience with neonatal rodent models of periventricular hematoma, severe hydrocephalus is a relatively uncommon occurrence (Balasubramaniam et al., 2006). The subarachnoid scarring of post-meningitis or post-hemorrhage hydrocephalus can be modeled by kaolin injection (Del Bigio, 2001).

The postnatal 21-day rat corresponds roughly to a 6-month human infant with regard to brain development,

Abbreviations: BS, *Bandeiraea* (Griffonia) *simplicifolia*; CGalT, ceramide galactosyltransferase; CNPase, 2,3-cyclic nucleotide phosphodiesterase; CSF, cerebrospinal fluid; DA, dopamine; DAB, diaminobenzidine; DOPAC, 3,4-dihydroxy-phenylacetic acid; GFAP, glial fibrillary protein; MBP, myelin basic protein; MR, magnetic resonance; NE, norepinephrine; PNPP, *p*-nitrophenyl-phosphorylcholine phosphocholine phosphodiesterase; SEZ, subependymal zone; 5-HT, serotonin.

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during which time cerebral myelination is developing rapidly (Jacobson, 1963; Romijn et al., 1991). Induction of hydrocephalus at this age is associated with delayed myelination in subsequent 1–4 weeks, which can be prevented by early diversionary shunting of CSF (Del Bigio et al., 1997a,b). The model has been used to test drug protection (Del Bigio and Massicotte, 2001; Khan et al., 2003a,b). Most studies of early onset hydrocephalus, corresponding to fetal-onset or premature infant-onset hydrocephalus in humans, have been conducted in the mutant H-Tx rat model, in which the ventricles begin to dilate shortly before birth (Boillat et al., 1997; Jones and Bucknall, 1988). Because there remain some uncertainties about the genetic explanations for brain maldevelopment in that model (Jones et al., 1999, 2001, 2004) and because hydrocephalus in premature human infants is of various etiologies, we wanted to characterize in detail early onset hydrocephalus in neonatal rats using a non-genetic model (Del Bigio, 2001). Only a few publications concerning this model exist (Chovanes et al., 1988; Cosan et al., 2001, 2002; Ishizaki et al., 2000; McAllister et al., 1985). Our goal is to describe in detail the sensorimotor, anatomical, histological, and biochemical alterations with respect to postnatal brain development. We hypothesized that early onset hydrocephalus in rats will be associated with delay in sensorimotor development and white matter damage that is dependent on ventricle size.

Methods

All animals were treated in accordance with guidelines set forth by the Canadian Council on Animal Care. The local animal use committee approved the experiments. All efforts were made to minimize suffering and the number of animals used.

Animal preparation

Sprague–Dawley rats were bred locally, and 9 litters (10–12 pups per litter) including 98 pups were used. Before kaolin induction, each litter was designated for euthanasia at 7 or 21 days, dictated by tissue needs and availability of the MR imaging system. Rats were placed on aluminum foil over ice for cold anesthesia on postnatal day 1. The neck was wiped with ethanol, and under aseptic conditions, a 28-gauge needle was inserted percutaneously into the cisterna magna (n = 70). Sterile kaolin suspension (0.02 ml; 250 mg/ml in 0.9% saline) was injected slowly to induce hydrocephalus. The pups were returned to their mothers after rewarming. They were housed in standard cages and provided with a normal 12-h day/night lighting schedule with free access to water and pellet food. For identification, numbers were marked on the backs with permanent marker and ear punches were made at 7 days. Along with the hydrocephalics, 8 intact controls and 3 sham-injected rats were studied at 7 days and 12 intact controls and 5 sham-injected rats were studied at 21 days.

Magnetic resonance imaging

Magnetic resonance (MR) studies were performed at 7 or 21 days using a Bruker Biospec/3 MR scanner equipped with a 21-cm bore magnet operating at a field of 7 T (Karlsruhe, Germany) to obtain T2-weighted images of the brain in the coronal plane (slice thickness 0.5 mm) (Del Bigio et al., 1997a, b). When possible, as dictated by availability of the imaging system, rats destined for 21-day survival were also imaged at 7 days. The areas of the lateral ventricles and cerebrum were measured (by computerized planimetry using locally developed software) in the rostral cerebrum at the level of the optic chiasm. Frontal horn size was expressed as a ratio determined by dividing the total area of the ventricles by the area of the cerebrum. Rats were categorized as having either mild or severe ventriculomegaly using a cut-off ratio of 0.25. This value was chosen after analyzing all images because it allowed division into two roughly equal groups for statistical comparisons. For logistical reasons, one 21-day-old pup used for behavioral and biochemical analyses did not have a final MR image, but at brain removal, the ventricles were grossly enlarged.

Behavioral assessment

Rats were weighed on days 7, 12, 14, 19, 20, and 21. To study early sensorimotor development, the following outcome measures were examined: ambulatory behavior, righting response, and negative geotactic reactions. These tests were previously validated (Altman and Sudarshan, 1975). The rats were always tested in the same order. Each of these tests was completed in triplicate and averaged. Observing the pups for directed head and forelimb movements on postnatal days 4–7 assessed early ambulatory behavior. Righting response was assessed on day 7 by placing a pup on its back and timing how long it took for it to return to prone position. Negative geotaxis was also assessed on day 7. Pups were placed on a 35° incline plane with head facing down the plane. The maximum time it took for the pup to turn around 180° so that head was facing up the plane was recorded. On postnatal days 10, 14, 17, and 20, forelimb grip strength was tested by timing the rats' ability to hang from a 1.5-mm diameter wire, elevated 40 cm above a soft sponge. The maximal time allotted was 12 s. Gait agility was assessed on day 20 using a rotating cylinder (diameter 8 cm; Economex, Columbus Instruments) in two separate trials. First, endurance was assessed at a constant speed of 5 revolutions per minute (rpm) for a maximum of 2 min. Second, we measured ability to stay on the cylinder while it accelerated at a rate of 0.1 rpm every second for up to 2 min. The outcome measures and testing protocols for rotating cylinder are the same as those described in juvenile rats with hydrocephalus (Del Bigio and Massicotte, 2001; Khan et al., 2003a,b). Finally, on day 20, after each pup had completed the rotating cylinder test, swimming speed was measured in a 20-cm-deep, 1.5-m straight channel; the rats performed three trials, separated by a 30-s rest. Learning and memory were assessed using the modified Morris water maze test in a 90-cm pool filled with 22°C water and containing a 13-cm round hidden platform as previously described (Del

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