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Reduced subventricular zone proliferation and white matter damage in juvenile ferrets with kaolin-induced hydrocephalus

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ABSTRACT

Hydrocephalus is a neurological condition characterized by altered cerebrospinal fluid (CSF) flow with enlargement of ventricular cavities in the brain. A reliable model of hydrocephalus in gyrencephalic mammals is necessary to test preclinical hypotheses. Our objective was to characterize the behavioral, structural, and histological changes in juvenile ferrets following induction of hydrocephalus. Fourteen-day old ferrets were given an injection of kaolin (aluminum silicate) into the cisterna magna. Two days later and repeated weekly until 56 days of age, magnetic resonance (MR) imaging was used to assess ventricle size. Behavior was examined thrice weekly. Compared to age-matched saline-injected controls, severely hydrocephalic ferrets weighed significantly less, their postures were impaired, and they were hyperactive prior to extreme debilitation. They developed significant ventriculomegaly and displayed white matter destruction. Reactive astroglia and microglia detected by glial fibrillary acidic protein (GFAP) and Iba-1 immunostaining were apparent in white matter, cortex, and hippocampus. There was a hydrocephalus-related increase in activated caspase 3 labeling of apoptotic cells (7.0 vs. 15.5%) and a reduction in Ki67 labeling of proliferating cells (23.3 vs. 5.9%) in the subventricular zone (SVZ). Reduced Olig2 immunolabeling suggests a depletion of glial precursors. GFAP content was elevated. Myelin basic protein (MBP) quantitation and myelin biochemical enzyme activity showed early maturational increases. Where white matter was not destroyed, the remaining axons developed myelin similar to the controls. In conclusion, the hydrocephalus-induced periventricular disturbances may involve developmental impairments in cell proliferation and glial precursor cell populations. The ferret should prove useful for testing hypotheses about white matter damage and protection in the immature hydrocephalic brain.

Introduction

Hydrocephalus is a common neurologic condition characterized by altered cerebrospinal fluid (CSF) flow with enlargement of ventricular cavities in the brain. The pathophysiology of hydrocephalus-induced brain damage is multifactorial, with primary destruction of periventricular axons due to gradual physical stretching and compression, ischemia, accumulation of metabolic waste products in the CSF (McAllister and Chovan, 1998), impaired blood flow, and calcium-mediated axonal cytoskeletal damage (Del Bigio, 1993, 2000, 2004). The histopathologic consequences of hydrocephalus are dependent on the age of onset, rate of ventricular enlargement, and the etiology (Del Bigio, 2001b; Hochwald, 1985).

The pathogenesis of brain damage in hydrocephalus has been elucidated by studies of human brains and through use of experimental animal models (da Silva Lopes et al., 2009; Del Bigio, 1993, 2001a, 2004; Del Bigio et al., 1994; Khan et al., 2006). The most commonly used method for induction of experimental hydrocephalus in a variety of species is injection of kaolin (aluminum silicate) into the cisterna magna (Dixon and Heller, 1932). Inflammation and fibrosis leads to an obstruction of the CSF pathways close to the fourth ventricle apertures and in the basal subarachnoid compartment. Research with rodents is limiting because their brains possess a small volume of white matter, which makes it difficult to study changes in the fate of oligodendrocytes and periventricular axons. Thus, it is essential to produce another model with an animal that has a complex brain, which is

Abbreviations: APP, amyloid precursor protein; CGalT, ceramide galactosyltransferase; CNPase, 2′3′cyclic nucleotide 3′phosphodiesterase; COX, cytochrome c oxidase; CSF, cerebrospinal fluid; DAB, diaminobenzidine; DG, dentate gyrus; ELISA, enzyme linked immunosorbent assay; GFAP, glial fibrillary acidic protein; GLM, general linear model; GPC-PP, glycerylphosphorylcholine phosphocholine phosphodiesterase; H&E, hematoxylin and eosin; ISVZ, inner subventricular zone; MBP, myelin basic protein; MR, magnetic resonance; NADH, nicotinamide adenine dinucleotide; NF, neurofilament; NRC, National Research Council; OSVZ, outer subventricular zone; P, postnatal day; PBS, phosphate-buffered saline; pNPP, *p*-nitrophenylphosphorylcholine; SDH, succinic dehydrogenase; SEM, standard error of the mean; SVZ, subventricular zone; TE, echo time; TR, recovery time; VZ, ventricular zone.

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more similar to humans. Furthermore, large animals with gyrencephalic brains are necessary for the preclinical demonstration of treatment efficacy in stroke (Fisher et al., 2009; (STAIR) STAIR Group, 1999). Given that the pathogenesis of hydrocephalus associated axon damage has overlap with stroke (hypoperfusion, oxidative mechanisms, calcium ion mediated effects), it is reasonable to expect that a similar study approach should be used.

In this regard, domestic ferrets (*Mustela putorius furo*) have been chosen for this study for several reasons. Ferrets have complex gyrencephalic brains similar to cats and dogs (all are in the order *Carnivora*) and can easily fit into narrow bore magnetic resonance (MR) imaging systems. Ferrets are born in a relatively immature state with a prominent ganglionic eminence, and therefore could be used to study models of post-hemorrhagic hydrocephalus associated with premature birth (Hayes et al., 2003). Much is already known about development of the ferret brain (Lockard, 1985; McSherry and Smart, 1986; Voigt, 1989). In addition, ferrets are easily handled and are amenable to behavioral testing for assessment of brain damage (Rabe et al., 1985).

Brain changes that include enlarged cerebral ventricles have been induced in ferrets using methylazoxymethanol, an alkylating neurotoxin that destroys dividing cells (Haddad and Rabe, 1980), and reovirus (Margolis and Kilham, 1969). However, because these two processes are primarily destructive in nature, they are not especially useful for understanding the consequences of ventricular dilatation. As has been done in kittens (McAllister et al., 1991), we have successfully induced hydrocephalus in postnatal day 14 ferrets using kaolin, which was monitored until about 56 days of age. Our goal is to fully characterize kaolin-induced hydrocephalus in juvenile ferrets by examining the neuropathological, behavioral, and cognitive deficits associated with this condition. We specifically want to address the hypothesis that early onset hydrocephalus alters white matter development.

Methods

Animals

Pigmented sable ferrets were obtained from Marshall Farms (North Rose, NY), as timed-pregnant jills or jills with litters. The kits remained with their mothers in enclosed kennels until postnatal day (P)56. Food and water were provided ad libitum; the kits begin to eat solid food around P30. The kennels were kept on a 12:12 h (6 a.m. – 6 p.m.) light–dark cycle, and the room temperature and relative humidity were 21–23 °C and ~55%, respectively. For identification, tattoos were imprinted on their paws. All animals were treated humanely based on the guidelines devised by the Canadian Council on Animal Care. The institutional animal ethics committee approved the experimental protocols. All efforts were made to minimize the number of animals used and ensure the least amount of suffering experienced.

Hydrocephalus induction

In pilot studies using anesthetized P2, P7, and P14 and adult ferrets, we assessed the efficacy of percutaneous injections of kaolin (aluminum silicate; Sigma-Aldrich Corporation, St. Louis MO) into the cisterna magna. Four young ferrets (two at P2 and two at P7) were injected with 10% kaolin, but all died shortly after injection from either a hematoma or failure to feed after injection. Careful anatomical dissection suggests that the high complication rate is related to the close proximity between the skull base and the first cervical vertebra, leaving little room for safe needle insertion. There was also a high rate of maternal abandonment of kits that were handled early. Young adult ferrets (~6 months) injected with kaolin ($n = 4$) were quite ill following the procedure and did not develop ventriculomegaly.

The following experiments utilized 8 litters of 13- or 14-day old ferrets (~6 per litter − total $n = 45$). For the kaolin injections, the ferrets ($n = 25$) were anesthetized by inhalation of isoflurane (2.5% in oxygen). With the dorsum of the neck shaved, flexed, and cleansed with chlorhexidine followed by 70% alcohol, a 27-gauge needle (shallow bevel "blunt" tip) attached to a 0.5 mL syringe was inserted percutaneously into the cisterna magna and 0.2 mL of 20% sterile kaolin suspension (250 mg/mL in 0.9% saline) was injected slowly under aseptic conditions. Littermate controls ($n = 20$) received sham injections of sterile saline solution. Animals were monitored while recovering from anesthetic and observed for indications of discomfort and/or signs of neurological impairment. Following recovery, they were returned to their mothers. They were monitored every 12 h for the next two days and received subcutaneous injections of buprenorphine analgesic to reduce potential pain and sterile 0.45% saline to prevent dehydration. Ferrets were weighed daily and observed for signs of neurologic impairment. Those experiencing severe impairment or weight loss were euthanized to prevent further suffering. Control and hydrocephalic ferrets were sacrificed at approximately P14, P21, P35, P42, P49, and P56 to examine developmental and neuropathological differences between the two groups at various ages.

Magnetic resonance imaging

Magnetic resonance (MR) studies were performed on the ferrets at various time points ranging from P8 to P56 to obtain images pre- and post-kaolin injections. Ferrets were imaged 2 days post-injection to ensure that hydrocephalus had been induced. Subsequently, several kits were imaged at P21 and each week thereafter until P56 to acquire at least 4 hydrocephalic and 4 age-matched control kits at each survival time. The ferrets were anesthetized using 5% isoflurane in O_2/N_2O and maintained at ~2.5% isoflurane in O_2/N_2O with a nose cone. Respiration and external body temperature were monitored during imaging using an MR-compatible small animal monitoring and gating system (SA Instruments, Inc., Stony Brook, NY). Temperature was maintained at 37 °C with a heating circulator bath (Thermo Scientific HAAKE, Karlsruhe, Germany). The coils used were custom-built inductively coupled quadrature RF volume coils (National Research Council (NRC) Institute for Biodiagnostics, Winnipeg, MB, Canada). The images were attained with a Bruker Biospec/3 MR scanner equipped with a 21 cm bore magnet operating at a field of 7 T (Karlsruhe, Germany) to collect T2-weighted images of the brain oriented in the coronal, horizontal, and sagittal planes (slice thickness 1.0 mm). The recovery time (TR) for the T2-weighted images was 16.65 ms, and the effective echo time (TE) was 80 ms. Initially, the young ferrets were placed in a supine position into a 33 mm coil during the procedure. As they developed and their heads and skulls grew, they had to be placed into a 38 mm coil. By P35, they had to be put into the scanner with a 48 mm coil. Concurrently, 10 coronal images were obtained at 2 mm apart from P14 until P35 capturing brain regions rostrally from the frontal horn to fourth ventricle caudally. From P42 onward, 12 coronal images were taken at 2 mm apart to encompass the frontal horn to the fourth ventricle, which were centered and matched brain regions from images obtained at younger ages.

The areas of the lateral ventricles and cerebrum were measured (using computerized planimetry with Marevisi software; NRC, Winnipeg, MB, Canada) in the rostral cerebrum at the coronal level immediately caudal to the optic chiasm; the lateral ventricle frontal horn size was calculated by dividing the ventricle area by the cerebrum area. The third ventricle size was calculated as a ratio of its maximum width to that of the cerebrum at the same coronal level. The cerebral aqueduct size was calculated as a ratio of its area to that of the midbrain. In addition, the cerebral aqueduct was examined for flow void phenomena, which occurs when there is a reduced or absence of signal fluid motion (Jack et al., 1987). The fourth ventricle size was calculated as a ratio of the hindbrain area (medulla + cerebellum).