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Is the ferret a suitable species for studying perinatal brain injury?

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ABSTRACT

Complications of prematurity often disrupt normal brain development and/or cause direct damage to the developing brain, resulting in poor neurodevelopmental outcomes. Physiologically relevant animal models of perinatal brain injury can advance our understanding of these influences and thereby provide opportunities to develop therapies and improve long-term outcomes. While there are advantages to currently available small animal models, there are also significant drawbacks that have limited translation of research findings to humans. Large animal models such as newborn pig, sheep and nonhuman primates have complex brain development more similar to humans, but these animals are expensive, and developmental testing of sheep and piglets is limited. Ferrets (*Mustela putorius furo)* are born lissencephalic and undergo postnatal cortical folding to form complex gyrencephalic brains. This review examines whether ferrets might provide a novel intermediate animal model of neonatal brain disease that has the benefit of a gyrified, altricial brain in a small animal. It summarizes attributes of ferret brain growth and development that make it an appealing animal in which to model perinatal brain injury. We postulate that because of their innate characteristics, ferrets have great potential in neonatal neurodevelopmental studies.

1. Introduction

Survival of preterm infants born at or before 28 weeks of gestation continues to improve, and currently, the majority of infants born as early as 24 weeks of gestation survive (Jarjour, 2015; Stoll et al., 2010). However, nearly 50% of survivors develop moderate to severe neurodevelopmental impairment (Jarjour, 2015; Gargus et al., 2009; Hintz et al., 2011; Ancel et al., 2011). Intraventricular hemorrhage (IVH), hydrocephalus, periventricular white matter (WM) injury and encephalopathy of prematurity are common complications of prematurity and contribute to these poor outcomes (Stoll et al., 2010; Volpe, 2009a). Neonatal inflammation due to maternal chorioamnionitis and postnatal infection can markedly exacerbate neonatal brain injury at all ages (Hagberg et al., 2012; Thornton et al., 2012; Volpe, 2011). While current treatments including antenatal steroids (Friedman and Shinwell, 2004), magnesium sulfate (Crowther et al., 2003), and postnatal caffeine (Schmidt et al., 2012) improve long-term outcomes, there is still great need for additional, more efficacious neuroprotective therapies. Relevant animal models are needed to not only delineate the pathophysiology of neonatal brain injury, but also to discover new therapeutic interventions to halt and/or reverse these processes.

Small and large animal models of preterm brain injury, hypoxic ischemic brain injury and stroke are currently used to understand mechanisms of injury. These models have several advantages and disadvantages, such as similarity to human brain development, cost of husbandry and technical applications. Ferrets (*Mustela putorius furo*) are domesticated animals, and they have been increasingly used in biomedical research as models of respiratory viral diseases (Ball, 2006; Belser et al., 2011) and gastrointestinal diseases (Alder et al., 1996). More recently they have been used to understand the pathophysiology of immature brain injury. Ferrets lend themselves well to the study of neurodevelopment and brain injury because they are born lissencephalic with a prominent ganglionic eminence, and later undergo postnatal cortical folding to form complex gyrencephalic brain (Reillo and Borrell, 2012), comparable to brain growth as seen in premature human infants (Barnette et al., 2009). Domestic ferrets have social communication skills comparable to dogs (Hernadi et al., 2012) suggesting that sophisticated neurodevelopmental assessments should be plausible in these species. This review describes the suitability of ferrets as species to study perinatal brain injury in humans.

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2. Animal models of neonatal brain injury

Historically, the most common animals used to study brain development have been rodents, rabbits and cats. Similar to humans, these animals undergo substantial postnatal brain development. Notable neonatal rodent models are the so-called Vannucci model of hypoxic-ischemic brain injury (Rice et al., 1981), the middle cerebral artery occlusion (MCAO) model of stroke (Ashwal et al., 1995), and the chronic hypoxia model (Schwartz et al., 2004; Salmaso et al., 2012). The classic Vannucci model induces unilateral brain injury in a postnatal day (P) 7 rat by unilateral carotid artery ligation followed by hypoxia (8% oxygen) for 90 min. Since its description, this injury model has been adapted to younger and older rat pups and to mice of various strains, demonstrating the variable vulnerabilities of rodent pups at different ages, and in different species (Northington, 2006). Both the P3 mouse (Dribben et al., 2009) and P3 rat brains (Lodygensky et al., 2014) are considered to have similar brain development as seen in 25-week premature human infant. The MCAO stroke model was initially used in P14–18 rats, but has since been adapted for use in P7 rats (Wen et al., 2004). The Vannucci and MCAO models create large unilateral injuries to both gray and white matter, but the MCAO model includes reperfusion as part of the injury. The chronic hypoxia model exposes animals to 10% oxygen from P3 to P11 and results in white matter injury (Back et al., 2006) and diminished neuronal growth (Schwartz et al., 2004). While similar changes are seen in some preterm infants, the mechanisms of injury are likely quite different since preterm infants do not experience chronic hypoxia. All of these models are limited in their ability to translate effectively to humans because of differences in brain complexity, white to gray matter ratios (Zhang and Sejnowski, 2000), and mechanisms of brain injury.

Rabbits exhibit slightly more complex brain organization compared to rodents, and several rabbit models of brain injury have been developed. They are altricial animals with immature brain at birth (Nicolas et al., 2011). They share the same hemochorial placenta to rodents, sheep and primates (Enders and Carter, 2004). A neonatal rabbit model of global hypoxia-ischemia created by sustained uterine artery occlusion at 70% and 75% of gestation *in utero* provides a reproducible model of cerebral palsy (Tan et al., 2005; Buser et al., 2010). There is also a rabbit model of IVH created by intraperitoneal glycerol injection of premature rabbits (Georgiadis et al., 2008). These models have moderate to high postnatal mortality rates. Furthermore, glycerol used to induce IVH may have direct cytotoxic effects unrelated to prematurity (Traudt et al., 2014). In comparison to rodents, rabbits do not have an extensive array of antibodies or other standard testing materials available for research. They also have limited behavioral assessments including motor tests (Tan et al., 2005; Yu et al., 2009; Georgiadis et al., 2008), and tests for short term memory and attention (Illa et al., 2013). They also have limited vascular access for monitoring and regular blood sampling (McArdle et al., 2010).

New animal models are being developed to reflect current understanding of the pathophysiology of preterm brain injury that includes varying combinations of inflammation (Kuban et al., 2014; Dammann and Leviton, 2014), hypoxia (Groner, 1997), hypoxia-ischemia (Stridh et al., 2013) and/or hyperoxia (Ritter et al., 2013). Models that demonstrate these effects have been created in immature rabbits (Balakrishnan et al., 2013), newborn kittens (Balasubramaniam and Del Bigio, 2006), dogs (Ment et al., 1982) and rodents (Burd et al., 2012; Dean et al., 2015). Small animal models have several advantages, especially rodents. Rodents have a relatively low cost of care and maintenance accompanied by an accelerated life cycle. Commercial dietary products and species-specific antibodies are readily available and methods of behavioral and electrophysiological testing are well established. Many genetically modified strains exist, and availability of a fully sequenced genome allows for development of therapeutic strategies with specific targeted pathways. Behavioral testing in rodents can include evaluation of motor control, coordination, learning, memory and sensorimotor gating as well as tests of braveness and social interaction (Kim et al., 2015; Ramani et al., 2013; Ten et al., 2004). There are also drawbacks to the use of rodent models. The rodent brain is lissencephalic with a much smaller proportion of white matter than is present in humans (Zhang and Sejnowski, 2000). The foci of neurogenesis and timing of myelination are different. These factors may be important when studying the effect of an early insult on later brain development. The rate of rodent maturation is quite accelerated relative to humans with each day of rat development corresponding to more than a week of human development (Clancy et al., 2001). Despite this, the timing of the cellular response to injury appears to be similar in both species. Thus as brain injury unfolds over hours to days, the developmental context changes differentially in rodents compared to humans; for example, as injury evolves from P7 to P10 in a rat, this time frame would roughly span 32 weeks to term in a human infant. Since the cellular and regional vulnerability of brain varies by developmental stage, the effect of brain injury and its repair may be quite different in rodents than humans (Balasubramaniam and Del Bigio, 2006). These important differences have limited the translation of therapeutic interventions to humans. Despite these shortcomings, these small animal models have formed the cornerstone of our current understanding of mechanisms of brain injury and the basics of neurodevelopmental pathways used to develop new therapies.

Larger mammals such as newborn pig (Iwata et al., 2007; Ezzati et al., 2014), fetal sheep (Castillo-Melendez et al., 2013; Gisslen et al., 2014; Bennet et al., 2007), and nonhuman primates (Inder et al., 2004; Beckstrom et al., 2011) are popular as models of neonatal brain injury because of their more complex brain structure. All of these species have long pregnancies and varying gestational ages when the fetal brain growth is similar to that of a human preterm infant (Clancy et al., 2001; Kim et al., 2014; Wassink et al., 2015; Griffith et al., 2012). Both sheep and piglets are precocious at birth (Butler et al., 2009; Karlsson et al., 2011). They can be chronically instrumented for physiologic measurements (Dalitz et al., 2003; Lemery et al., 1995) and can tolerate repeated blood sampling (Ferrara et al., 1995). However there are very few motor (Duberstein et al., 2014), cognitive and behavioral tests (Sullivan et al., 2013; Friess et al., 2007; Greiveldinger et al., 2011) available to monitor long-term effects of brain injury. Nonhuman primates, in particular, are useful to understand neurobehavioral outcomes of diseases (Mack et al., 2003; Jacobson Misbe et al., 2011) and effects of therapeutic interventions. However, disadvantages such as their higher cost, complicated husbandry (Alonso-Alconada et al., 2015; Nitsos et al., 2006; Inder and Neil, 2005) and large size make them more suitable for late preclinical trials. The use of these valuable animals requires conscientious resource management. Ferrets pose an attractive alternative with less cost but similar cortical development and complex behavior to large animal models (Sawada and Watanabe, 2012). They have great potential as neurodevelopmental research models. The pros and cons of ferrets in comparison to small and large brain injury models are shown in Table 1.

3. Ferrets (*Mustela putorius furo*)

Ferrets are carnivores belonging to the family *Mustelidae*, which includes weasels, otters and minks. The European ferret (*Mustela putorius furo*) has been domesticated for many years and is generally considered a hardy animal with its tolerance of anesthesia and surgical procedures being well described in the veterinary literature (Cantwell, 2001; Brown, 2006; Siperstein, 2008). It is