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Review

The great barrier belief: The blood–brain barrier and considerations for juvenile toxicity studies

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ABSTRACT

Juvenile animal studies can be warranted to support the development of pediatric medicines. Drugs acting on the CNS or those which penetrate into the brain merit particular attention. The blood-brain barrier is functionally mature at birth, but undergoes functional postnatal modulation to provide a suitable microenvironment for the developing brain. In the past, dosing in rat juvenile studies has often commenced at 4 or 7 days of age. However, rodents are very neurologically immature at birth compared with humans. We suggest that dosing of rat pups below two weeks of age is generally not warranted for the assessment of pediatric drugs. In the rare circumstances where exposure of younger rats is required to address a particular concern (e.g., an indication in preterm babies), consideration should be given to likely misleading signals of toxicity arising from high brain penetration of the drug, which may not be predictive for the human.

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1. Introduction

It is widely accepted that the developing brain is more vulnerable to drugs and toxins than the adult brain [1]. This increased vulnerability may be due to increased sensitivity of the immature brain to the effects of the chemical, such as apoptotic neurodegeneration induced by NMDA blockade in 7-day old rats [2], but within the context of safety testing is more often associated with increased brain levels of the toxicant. Many chemicals and medicines have been found to reach higher brain concentrations for a given systemic exposure in neonatal humans and animals than in adults [3]. Valproate for example was shown to reach three-times greater concentrations in the neonatal rabbit brain than in the adult brain following injection into a carotid vein [4]. Likewise, for the neurotransmitter, γ-aminobutyric acid (GABA) the brain:plasma ratio is twice as high in the neonatal rat than in the adult [5]. This increased brain exposure in the neonates has often wrongly been attributed to immaturity of the blood–brain barrier (BBB) [6]. It has been known for some time that a functional barrier is already established at birth [7,8]. During the course of pre- and postnatal development, the barrier mechanisms are dynamically modulated to provide the necessary microenvironment for the developing brain [9]. The brain uptake of amino acids is high in the neonate, to satisfy the greater nutritional demands of the developing brain, and then declines

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towards adulthood [10]. This decline in uptake with postnatal age was initially postulated to be due to the ongoing myelination of neurons contributing to a greater lipidic barrier around the blood vessels, but this was discounted when the extent of the decline was shown to be independent of the lipophilicity of the molecule [11]. Most complex molecules and many drugs that enter the brain from the plasma do so via the cerebrospinal fluid (CSF) at the choroid plexus [5,12]. The influx and efflux transporters at the blood-CSF interface are well developed at birth [13].

Thus, many drugs and potential neurotoxicants enter the brain via the active transport systems that have evolved to transport nutrients and other molecules essential for the maintenance and development of the CNS. The question of relevance to the design of pediatric safety studies is not from what age the BBB "closes", but rather to what extent the drug gains access into the developing brain at various stages of development, both in the human and in model species. The increased susceptibility of the neonate to the neurotoxicity of drugs is thus not due to immaturity of the BBB, but is rather potentiated by the already highly active transport systems [14].

Higher brain penetration of chemicals in neonates can also be due to developmental features not intrinsic to the central nervous system (CNS) that influence plasma pharmacokinetics, such as differences in volume of distribution related to body composition (i.e. overall greater water content and lower lipid partition), reduced blood plasma protein binding capacity and reduced renal flow. The apparent porosity of the brain may also be influenced by physiological differences in the juvenile, such as a greater brain mass relative to body weight, greater blood flow to the brain and reduced flow of the CSF through the cerebral ventricles.

The BBB is a multicellular and highly selective permeability barrier system that protects the brain tissue from exposure to potential toxicants, either directly from the blood or via the CSF [15]. Various cell types, cellular interfaces with tight junctions, extracellular matrix components and transporter mechanisms comprise the BBB.

A major consideration in designing a toxicology program in support of pediatric safety is how brain concentrations of a drug are likely to differ between children of various ages and the adult. In some cases, pediatric-specific hazards need to be assessed in a juvenile animal study. The rat is a preferred model for juvenile toxicity testing [16]. Rodents are very immature at birth compared with other mammalian species, particularly with respect to CNS development [17]. It is not surprising, therefore, that the neonatal rat has proven to be especially vulnerable to CNS toxicity of drugs. In several small molecule drug-development programs, we found that 4- or 7-day old rats did not tolerate doses of drugs that are without effects in 10- or 14-day old rats (unpublished). Systemic exposure of the newborn animal may occur following direct dosing of the pups or may be transferred from the mother, prenatally across the placenta and/or postnatally via the milk (see bitopertin example below).

The brain is a likely target organ of toxicity for any drug that crosses the BBB in the neonate. The vulnerability of the developing CNS lies in the continued developmental processes (cell division, differentiation, migration and synaptogenesis) to form the adult-like complex neuronal network accompanied and guided by surrounding glia cells [14]. An understanding of the critical windows of vulnerability of the developing brain is necessary. Predicted CNS exposure to a drug at different ages can then be compared with the timing of the critical periods of brain development to identify potential risks [17,18]. The much greater complexity of the human brain hinders the comparison of the chronology of CNS development between species, with different brain regions developing at different relative times. For instance, rodents do not possess a dorsolateral pre-frontal cortex, which is implicated in higher cognitive functions in the human.

The rat brain is very immature at birth. Neurogenesis is still ongoing in the newborn rat in several regions that are already relatively mature in the human, such as the mesencephalic tectum, striatum, amygdala, neocortex and limbic cortex [18]. With respect to neurogenesis in the later-developing regions –e.g. cerebellum, hippocampus and olfactory bulb-, the 16–19 day old rat is equivalent to a newborn human [19]. The degree of maturation of the cerebral cortex in a full-term human newborn is only reached in the rat by post-partum day (PND) 12–13 [20], while the development of the cerebral white matter in the rat on PND2 is equivalent to that of a premature baby[21]. Myelinogenesis commences during the first trimester of gestation in humans, but is mainly postnatal in the rat [18]. In the human, proliferation of synapses starts in mid-gestation, with a spurt immediately after birth to reach numbers approximately 50% higher than in the adult by two years of age [28,22]. The density of synapses in the rat brain remains low up to PND10 and then shows a spurt to reach adult numbers by about PND30 [22].

2. Morphological features of the BBB

Diffusional resistance between the blood-CNS interface is provided by tight junctions. At least four distinct barrier structures have been described so far: 1) blood-brain barrier proper to the cerebral vasculature: consisting of luminal tight junctions (zonulae occludentes) between the endothelial cells of cerebral blood vessels, 2) blood-CSF barrier to the choroid plexus: consisting of apical tight junctions between epithelial cells in the choroid plexus, 3) brain-CSF barrier to the pia arachnoid (meningeal barrier): consisting of tight junctions between cells of the arachnoid membrane, and 4) CSF-brain ventricular barrier: consisting of ependymal cells within the ventricles [14,15,23], see Fig. 1. These physical barriers are impermeable to proteins and other large molecules from early embryonic stages [14]. As a result, only small hydrophobic molecules which can cross cellular membranes and very small hydrophilic molecules can reach the brain by diffusion under normal conditions.

Recruitment of pericytes to completely cover the developing CNS vessels is critical for the formation and functional maintenance of the BBB [24,25]. Astrocytes play a role in the control and maintenance of the mature BBB, but are only recruited perinatally in the human or postnatally in the rat, after the barrier is operational [26,27].

In rats, a BBB of tight junctions as formed in-utero, but has a less complex three-dimensional appearance at birth than in the adult [28]. Also, a significant proportion of the capillaries form postnatally within the first three weeks after birth in the rat. The morphological appearance of the tight junctions varies between the different brain regions at various ages [29,30], thus contributing to the divergent results and views published on this subject.

3. Functional features of the blood-brain and blood-CSF barriers

The BBB is formed by the physical barrier of tight junctions, accompanied by a transport barrier comprising membrane enzymes, transporters and vesicular mechanisms [15]. As described above, the junctions between the cells making up the blood brain barrier are tight and so only very small hydrophilic molecules can pass and larger drugs must pass through the cellular membranes to reach the brain tissue. When a concentration gradient exists, molecules which can partition easily in and out of membranes from the aqueous extracellular and intracellular spaces will equilibrate. This process of passive diffusion is the dominant one for lipophilic, permeable molecules. For molecules with poor membrane parti-