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Nonhuman Primate Models and Developmental Neuronal Toxicity

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The existing data from research using rodent models have implicated some drugs as being toxic to the developing brain, even causing cognitive deficits in later life. However, there are no data providing evidence that the clinical use of these drugs is associated with signs of developmental neurotoxicity. This Editorial focuses on how use of the developing nonhuman primate, when combined with biochemical, pathological, pharmacokinetic, dynamic molecular imaging approaches and cognitive assessments, might provide a bridging model and, thus, serve to provide the most expeditious approach toward decreasing the uncertainty in extrapolating pre-clinical data to the human condition.

Early-life stress has been shown in both preclinical and clinical studies to cause neuroanatomical and biological alterations and disruptions in homeostasis. These alterations can lead to dysfunctions in critical regulatory systems and concomitant increases in risk for the development of pathology. Our goal in writing this Editorial is to highlight ways in which preclinical research models can inform clinical interventions and vice versa. Because of the complexity and temporal features associated with the normal course of brain maturation, the developing nervous system is likely to be much more susceptible than the mature brain to neurotoxic insults. The study of neurodevelopmental toxicology has great potential for helping to advance our understanding of brain-related biological processes, including neuronal plasticity, degeneration/regeneration, differentiation, toxicity and even therapeutic efficacy [1].

Recently described developmental neurotoxic insults involve the apoptotic cell death of neurons in the rodent brain following developmental exposure to sedatives and general anesthetics, such as ketamine and some inhalation anesthetics [2-5]. When these compounds are administered to the neonatal rat or mouse, a rapid and significant increase in apoptosis occurs in several brain regions [6]. Because of obvious concerns, it is not possible to thoroughly explore this kind of adverse anesthetic effect on neurons in human infants or children, nor is it possible to obtain relevant dose-response or time-course data about the potential sedative/anesthetic-induced neuronal cell death and associated behavioral deficits in humans.

The nonhuman primate is an animal model that has proved invaluable for informing aspects of human physiology, pathology, pharmacology, toxicology, and systems biology. No other commonly used research animal has a functional fetal-placental unit, a propensity for single births and a fetal-to-maternal weight ratio comparable to that of humans. Due to the complexity of the primate brain, the monkey is often the animal of choice for neurotoxicology experiments and given the protracted period of brain development the monkey is arguably the very best model for studies of developmental neurotoxicity. The phenomenon of interest in the present discussion (anesthetic-induced neuronal cell death in the brain) has also been previously observed in the nonhuman primate, *Macaca mulatta* [7,8]. Thus, the relevance of the sedative/anesthetic-induced neuronal cell death observed in rodent models to children is inferred because similar effects occurs the developing nonhuman primate.

This Editorial discusses several advantages for using the developing rhesus monkey in addressing critical issues related to the topic of pediatric sedation/anesthesia. These include the relationships between drug-induced neurotoxicity and developmental stage at time of exposure and how imaging tools might be combined with complex behavioral tests to provide opportunities to study the effects of drug exposure during development of important brain functions such as learning and memory.

Characteristics of Sedative/Anesthetic-Induced Damage in the Developing Monkey Brain

One great advantage of monkey models is that the anatomical and functional complexity of their CNS facilitates the interpretation of data with respect to the extrapolation of findings to humans. The first report regarding neuronal cell death in nonhuman primates exposed perinatally to anesthetics was published in 2007 [7]. This study focused on the representative general anesthetic, ketamine (a non-competitive NMDA receptor antagonist), which was administered as an intravenous infusion at doses sufficient to produce a light surgical plane of anesthesia [7]. The neurotoxic effects of these ketamine exposures were examined several hours after the end of the infusions, based on the hypothesis that ketamine induces an up-regulation of the NMDA NR1 receptor subunit, causing neurons to be more vulnerable to the excitotoxic effects of endogenous glutamate after ketamine has been cleared from the system. A 24 hours ketamine infusion was shown to produce a large increase in the number of TUNEL-positive cells in PND 5 monkey infants. Numerous darkly stained TUNEL-positive cells exhibiting the typical nuclear condensation and fragmentation indicative of enhanced apoptotic cell death were observed. The TUNEL assay relies on the detection of fragmented DNA strands. Consistent with ketamine's effects [7,8], an 8-hour exposure to a combination of inhaled anesthetics [70% nitrous oxide (N_2O) + 1% isoflurane (ISO)] also induced neuronal cell death in the PND 5 monkey brain that was primarily restricted to the cortical brain regions, especially in layers II and III of the frontal cortex. In addition to the frontal cortex, enhanced neuronal degeneration as evidenced by increased numbers of caspase 3-, silver- and Fluoro-Jade C-positive neuronal profiles was also observed in the temporal gyrus and hippocampal areas [9]. At the electron microscopic (EM) level, direct evidence of increased neuronal cell death in infant monkeys treated with anesthetics (either ketamine or the combination of inhaled anesthetics) was confirmed by the observation of cells exhibiting representative nuclear condensation and fragmentation (apoptosis) and necrotic characteristics, including

neuronal mitochondrial and cell body swelling [7,9]. It should be noted that the cellular pattern or topography and the nature of anesthetic-induced neurodegeneration seen in developing monkeys is different from that reported for developing rodents [4,5,10]. The data demonstrate that anesthesia-induced neuronal cell death in the neonatal monkey is both apoptotic and necrotic in nature. However, EM and other biochemical and morphological observations in the developing rat showed only the typical nuclear condensation and fragmentation (*in vivo and in vitro*) indicative of the advanced stages of apoptosis, not necrosis. These observations indicate that the potential toxicological consequences of prolonged anesthetic exposure in primates during development may be far more serious than that produced in rodents.

Anesthetic-Induced Neurotoxicity and Developmental Stage in Developing Monkey

The degree to which the nervous system is resistant to neurotoxic insults is highly dependent upon the stage of development. Because the brain growth spurt in both human and nonhuman primates extends over a much longer time period than in the rat, matching the timing of a developmental event in humans and nonhuman primates is less problematic than matching the same between primates and rodents. In addition to PND 5 monkeys, ketamine-induced neuronal degeneration was assessed in gestational day (GD) 122 and PND 35 monkeys [7]. As seen in the PND 5 monkeys, GD 122 fetuses showed clear ketamine-induced neuronal cell damage, whereas PND 35 monkeys did not. GD 122 fetuses and PND 5 infant monkeys, thus, are more sensitive to ketamine-induced cell death than PND 35 monkeys, when less synaptogenesis is occurring. Although a complete understanding of neuronal cell sensitivity to ketamine in the primate is not possible from these few early studies, it is apparent that rhesus monkeys are sensitive during the last 25% of gestation (term is 165 days) to sometime before PND 35. Equating relative stages of development between human and animal models is critical for the extrapolation of safety assessment data. It is generally believed that the nonhuman primate fetus (especially that of the rhesus monkey) and the human fetus are more similar in stage of maturation at birth as compared to rats that are relatively immature at birth. For example, both humans and rhesus monkeys are born with their eyes open at birth, whereas newborn rat pups are not. At PND 7 the rat pup is more similar in maturation to a monkey late in gestation than to an infant monkey. According to a recent review [11], the GD 123 monkey fetus is roughly equivalent to a GD 199 human fetus as determined by cortical development, and both are in the range of 75–80% of normal term. Also, NMDA receptor binding sites are present in the human fetal brain by GD 115, increase until GD 140–150, and then decrease slightly by GD 168–182 [12] and the localization of NMDA receptors in monkey cortex is similar to that observed in humans [13].

Anesthetic-Induced Brain Damage and Associated Physiological Parameters and Pharmacodynamic Outcomes in the Developing Monkey

For any animal model it is essential to monitor and control physiological parameters. As expected, these parameters are carefully controlled during pediatric sedation and anesthesia but the can be very difficult to control in rodent models, primarily because of their small size. The nonhuman primate, thus, provides a model that is ideal for these types of experiments. During anesthesia, all physiological parameters including percent oxygen saturation, exhaled carbon dioxide, body temperature, heart rate, blood pressure, glucose, and hematocrit can be monitored and maintained within normal ranges in the same manner as in the pediatric clinic. Because prolonged hypoperfusion can lead to cerebral hypoxia and ischemia-related cell death, it is necessary to maintain normal blood pressure and oxygen saturation [7,8,14] and this was readily accomplished in our nonhuman primate studies [7,14].

As expected, plasma ketamine concentrations are related to neuronal cell death in a dose-related fashion with higher doses causing more death. In perinatal monkeys, steady-state plasma ketamine concentrations of 10-25 µg/ml were achieved during prolonged periods (up to 24 hours) of anesthesia. While these plasma levels of ketamine are necessary to maintain anesthesia in the rhesus monky model, it is important to note that these levels are some 5-7 times higher than those required for human infants. It is also of interest to note that monkeys at different stages of development require different ketamine plasma concentrations to maintain anesthesia. For example, PND 35 monkeys required a higher plasma concentration of ketamine to maintain the same level of anesthesia as PND 5 monkeys. Another important finding was that even though the plasma concentrations of ketamine were highest in the PND 35 monkeys, there was no evidence of increased neuronal cell death, whereas in PND 5 animal's neuronal cell loss was significant even at lower plasma ketamine concentrations [7,14].

The Use of a Nonhuman Primate Model to Decrease the Uncertainty in Extrapolating Pre-Clinical Data to the Human Condition

Evidence in support of a correlation between surgery and subsequent neurophysiological changes has accumulated [15-17]. The use of a nonhuman primate model to decrease the uncertainty in extrapolating pre-clinical data [7,8,14] to the human condition (e.g. peri-operative neurotoxicity) continues to garner considerable interest among anesthesiologists and toxicologists, with growing recognition to be anticipated from surgeons and neonatologists. A host of mechanistic studies have been completed or are underway which have been helpful in providing a rationale for the overall concern over anesthetic and sedative-induced neurotoxicity. These studies have been and will be instrumental in teasing apart the causalities, refining hypotheses, developing alternative or protective measures and suggesting clinical strategies for assessing the phenomena in children. Such studies have ranged from cell culture to histopathology to animal behavioral studies – including the nonhuman primate [7,18-20]. To date, data from both rodents and nonhuman primates have demonstrated neurotoxic effects of anesthetic drugs on the developing brain that are associated with later deficits in important cognitive functions including learning, the ability to perform simple visual discriminations, motivation and speed of psychomotor processing [18]. There are, however, currently no clinical data providing evidence that the use of anesthetics during the perinatal period is associated with signs of developmental neurotoxicity or subsequent cognitive deficits in humans. It is essential to continue studies in nonhuman primates in order to obtain valuable information on the time course and severity of observed deficits. It will also be necessary to determine whether injured brain tissue can recover with no or minimal loss of function, or whether injured brain tissue can be protected from sedative/anesthetic-induced injury by the co-administration of anti-oxidants or other agents. Shorter durations of anesthesia have been shown to cause less or no cell death in monkeys: whether exposures to anesthetics will cause cell death in humans is still unknown, but it is likely that shorter exposures will have less impact than longer exposures. Although drug combinations are commonly used in pediatric procedures, there is a huge data gap concerning the neurodegenerative effects associated with sedative/anesthetic and other drug combinations.

A growing body of data indicates that molecular imaging with isotope-labeled biomarkers (radio-tracers) may help to detect neurotoxicity in infants, young and adult animals [21,22]. The high-resolution positron emission tomography scanner (microPET) can provide *in vivo* molecular imaging at a sufficient resolution to resolve both major structures and neuronal activities in the nonhuman primate brain. To determine whether prolonged sedative/anesthetic exposure during development is associated with subsequent long-term cognitive deficits, drug-induced neurodegeneration can be explored by monitoring changes in the uptake (binding) of radiotracers (e.g., [18F]-Peripheral Benzodiazepine Receptor ligand, a biomarker of neurotoxicity- and gliosis), in specific regions of interest in the monkey brain. In addition, Operant Test Battery tasks [23], including those for assessing aspects of learning, motivation, color and position discrimination, and memory can be useful tools in longitudinal assessments and in delineating the time course of cognitive performance deficits and underlying biochemical changes.

Summary

Information regarding risks associated with pediatric drugs is abundant in the animal literature, but philosophical, methodological and experimental differences among species, sources and models make direct comparison with humans difficult. It has been proposed that pediatric sedative/anesthesia-induced neurotoxicity depends on the amount (dose) given, the duration of the exposure, the route of administration, the receptor subtype activated, and the stage of the neural development at the time of exposure. These factors are important because they can help identify thresholds of exposure for producing neurotoxicity in the developing nervous system. There are yet many questions to answer before the findings of pediatric drug-induced neurotoxicity observed in animals can be related to effects in humans: however, the use of a nonhuman primate model combined with molecular imaging tools and dynamic behavioral tests might provide the most expeditious approach toward decreasing the uncertainty in extrapolating pre-clinical data to the human condition. Thus, further research in the nonhuman primate is urgently needed to determine which agents and procedures are likely to incur the greatest risk for subsequent brain dysfunction for both the very young and the elderly. In addition, the threshold doses and exposure durations necessary for safe and effective treatment, as well as possible protective strategies must be determined.

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References

1. Patterson TA, Schnackenberg BJ, Slikker W, Wang C (2011) Systems Biology Approaches to Neurotoxicity Studies during Development. Developmental Neurotoxicology Research: Principles, Models, Techniques, Strategies and Mechanisms. Wiley-Blackwell: 25-38.

2. Johnson KM, Phillips M, Wang C, Kevetter GA (1998) Chronic phencyclidine induces behavioral sensitization and apoptotic cell death in the olfactory and piriform cortex. J Neurosci Res 52: 709-722.

3. Shi Q, Guo L, Patterson TA, Dial S, Li Q, et al. (2010) Gene expression profiling in the developing rat brain exposed to ketamine. Neuroscience 166: 852-863.

4. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, et al. (2003) Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat rain and persistent learning deficits. J Neurosci 23: 876-882.

5. Scallet A, Schmued LC, Slikker W, Grunberg N, Faustino PJ, et al. (2004) Developmental neurotoxicity of ketamine: morphometric confirmation, exposure parameters, and multiple fluorescent labeling of apoptotic neurons. Toxicol Sci 81: 364-370.

6. Young C, Jevtovic-Todorovic V, Qin YQ, Tenkova T, Wang H, et al. (2005) Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. Br J Pharmacol 146: 189-197.

7. Slikker W, Zou X, Hotchkiss CE, Divine RL, Sadovova N, et al. (2007) Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol Sci 98: 145-158.

8. Zou X, Patterson TA, Divine RL, Sadovova N, Zhang X, et al. (2009) Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. Int J Dev Neurosci 27: 727-731.

9. Zou X, Liu F, Zhang X, Patterson TA, Callicott R, et al. (2011) Inhalation anesthetic-induced neuronal damage in the developing rhesus monkey. Neurotoxicol Teratol 33: 592-597.

10. Zou X, Sadovova N, Patterson TA, Divine RL, Hotchkiss CE, et al. (2008) The effects of L-carnitine on the combination of inhalation anesthetic-induced developmental neuronal apoptosis in the rat frontal cortex. Neuroscience 151: 1053-1065.

11. Clancy B, Finlay BL, Darlington RB, Anand KJ (2007) Extrapolating brain development from experimental species to humans. Neurotoxicology 28: 931-937.

12. Haberny KA, Paule MG, Scallet AC, Sistare FD, Lester DS, et al. (2002) Ontogeny of the N-methyl-D-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. Toxicol Sci 68: 9-17.

13. Huntley GW, Vickers JC, Morrison JH (1997) Quantitative localization of NMDAR1 receptor subunit immunoreactivity in inferotemporal and prefrontal association cortices of monkey and human. Brain Res 749: 245-262.

14. Hotchkiss CE, Wang C, Slikker W Jr (2007) Effect of prolonged ketamine exposure on cardiovascular physiology in pregnant and infant rhesus monkeys (Macaca mulatta). J Am Assoc Lab Anim Sci 46: 21-28.

15. Biedler A, Juckenhofel S, Larsen R, Radtke F, Stotz A, et al. (1999) Postoperative cognition disorders in elderly patients. The results of the "International Study of Postoperative Cognitive Dysfunction" ISPOCD 1. Anaesthesist 48: 884-895.

16. Johnson T, Monk T, Rasmussen LS, Abildstrom H, Houx P, et al. (2002) Postoperative cognitive dysfunction in middle-aged patients. Anesthesiology 96: 1351-1357.

17. Canet J, Raeder J, Rasmussen LS, Enlund M, Kuipers HM, et al. (2003) Cognitive dysfunction after minor surgery in the elderly. Acta Anaesthesiol Scand 47: 1204-1210.

18. Paule MG, Allen RR, Liu F, Zou X, Hotchkiss C, et al. (2011) Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol 33: 220-230.

19. Xie Z, Tanzi RE (2006) Alzheimer's disease and post-operative cognitive dysfunction. Exp Gerontol 41: 346-359.

20. Rizzi S, Ori C, Jevtovic-Todorovic V (2010) Timing versus duration: determinants of anesthesia-induced developmental apoptosis in the young mammalian brain. Ann N Y Acad Sci 1199: 43-51.

21. Zhang X, Paule MG, Newport GD, Zou X, Sadovova N, et al. (2009) A minimally invasive, translatable biomarker of ketamine-induced neuronal death in rats: microPET imaging using 18F-annexin V. Toxicol Sci 111: 355-361.

22. Zhang X, Paule MG, Newport GD, Sadovova N, Berridge MS, et al. (2011) MicroPET imaging of ketamine-induced neuronal apoptosis with radiolabeled DFNSH. J Neural Tansm 118: 203-211.

23. Paule MG (2001) Validation of a behavioral test battery for monkeys. In: Methods of Behavioral Analysis in Neuroscience. Buccafusco JJ (Ed.) CRC Press LLC, Boca Raton, FL: 281-294.