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The Use of Animal Models for the Study of FASD

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Abstract

Considerable efforts to educate women not to abuse alcohol during pregnancy have failed to reduce the incidence of fetal alcohol syndrome. Therefore, other approaches to limit the impact of prenatal alcohol abuse are under consideration, including the development of preventive and interventional strategies. To provide opportunity for these strategies to be as successful as possible, it is also important to improve methods of identification of affected children. Because of the practical and ethical limitations of utilizing human subjects in prenatal alcohol exposure research, investigators have addressed questions utilizing other biological platforms. This presentation will address more specifically the use of animal models to address three areas of research: the use of animal models to address basic questions about alcohol exposure during development, the use of animal models to improve the identification of affected individuals, and the use of animal models to develop approaches to reduce the impact of prenatal alcohol exposure. The various animal model systems that have been used to study FASD, each with their own specific strengths, have provided new findings that have been successfully extrapolated to human subjects resulting in advancement of the research field and our understanding of FASD.

Keywords

Fetal Alcohol Spectrum Disorders (FASD); fetal alcohol syndrome; alcohol teratology; animal models; ethanol; birth defects; prenatal alcohol exposure

INTRODUCTION

Observational studies in humans led to the identification of the association between prenatal alcohol exposure and neurodevelopmental disorders (Lemoine et al., 1968) and were important in leading to the first description of fetal alcohol syndrome (FAS) (Jones and Smith, 1973). Succeeding observational studies resulted in the identification and characterization of the comprehensive spectrum of disorders caused by prenatal alcohol exposure, now referred to as fetal alcohol spectrum disorders (FASD) (Riley and McGee, 2005, Mattson et al., 1998, Sampson and Streissguth, 1997). The first studies utilizing animal models of human prenatal alcohol exposure confirmed the relationship between prenatal alcohol exposure and the disorder that was identified in human observational studies (Abel and Dintcheff 1978, Chernoff 1977, Randall et al., 1977). The paucity of autopsy reports from FASD children, the absence of unequivocal alcohol exposure documentation and the inability to be able to eliminate the possibility of confounds including environment, other substances of abuse, and nutrition made these early animal

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studies both necessary and important. As a result of human observational studies and confirmative experiments in animal models of FASD, alcohol is now widely accepted by the scientific community and the public as a teratogen. Prenatal alcohol exposure is acknowledged as the leading cause of mental retardation in the Western world (Abel and Sokol, 1986). The estimated incidence of the full range of effects of prenatal alcohol exposure is 1% of live births (Sampson et al., 1997, May and Gossage 2001, O'Leary, 2004) and the estimated cost of FASD in the United States is $6 billion per year (Lupton et al., 2004). In light of the high incidence and cost, extensive efforts have been made to educate women about the dangers of drinking during pregnancy, yet the incidence of FAS remains essentially unchanged (Caetano et al., 2006). An estimated 120 million women in the United States drink alcohol and 10.1% continue to drink even after learning they are pregnant (CDC, 2002). Because the incidence of FASD has not declined and the societal cost is high, investigators are exploring more effective ways to identify affected individuals and ways to prevent or mitigate these disorders utilizing animal models.

While studies in humans have been very important in identifying the spectrum of disorders, the studies necessary to improve identification and how to prevent or ameliorate the actions of prenatal alcohol exposure are in many cases not possible in human subjects because of the potential for harming subjects, the limited ability to evaluate structural and functional damage and the difficulty in controlling variables in human subjects. Human studies must depend on unreliable self-reporting of alcohol consumption and have to contend with the potentially confounding effects of other drugs or tobacco that heavy drinkers often use concurrently with alcohol. In addition, controlling the variables of nutrition and genetics is difficult to impossible in human studies. Because of these limitations, investigators are performing studies in other biological platforms.

Investigators have for sound reasons utilized many different biological platforms to study different questions concerning prenatal alcohol exposure ranging in biological complexity from single cells to animal models that are very similar phylogenetically to humans. Cell and tissue culture experimental platforms are attractive because of their simplicity; experimental conditions are manipulated by controlling the tissue culture environment and thus the responses to the experimental conditions can be concluded to be due to actions on the specific cell or cells in culture. In addition, cell and tissue culture systems can allow for faster and less costly studies compared to those conducted in whole animals where time, space and material costs are greater. However, limitations to this approach are that cells in culture may not behave as they do in the whole organism and many of the disabilities caused by prenatal alcohol exposure are not characterized at the single cell level but instead must be studied at the whole organism level in order to appreciate the structural and or functional damage. In addition, the damaging effects of prenatal alcohol exposure depend on an exposure period during development that cannot be modeled in cell culture. Further, any identified action or effective protective action seen at the cellular level must be confirmed to be present at the more complex whole organism level. In addition, preventative or ameliorative measures must be shown to cause no harm in the whole organism.

Because of these issues, animal models of FASD are utilized to study basic mechanistic questions, protective or ameliorative strategies and to develop better tools to screen for individuals exposed to alcohol prenatally or that express the developmental damage. However, an acceptable animal model of FASD must meet certain criteria for the results of the studies to accurately reflect what happens in humans. The first criterion is that the animal model must express a sufficiently similar functional or structural disorder in response to alcohol exposure during development that is expressed in humans as a result of prenatal alcohol exposure. Meeting this criterion are animal models that range from being as organismally simple as helminths to those that are most organismally human-like, non-

human primates. The second criterion is that the mechanism of action by which the disorder is caused must be similar in humans. An example of a model that might fail this criterion would be one where the disorder under study is manifested only when alcohol exposures are so high that there is concern that the alcohol action may be mechanistically different than in humans that are exposed to far lower doses. Extensive evidence supports the conclusion that alcohol acts by different mechanisms depending on the dose, pattern of exposure and timing of exposure relative to fetal development (Goodlett et al., 2005). If these two criteria are met then conclusions from valid studies can potentially be rationally extrapolated to FASD in humans. The remainder of this presentation will address more specifically the use of animal models to address three areas of research: **1)** the use of animal models to address basic questions about alcohol exposure during development, **2)** the use of animal models to improve the identification of affected individuals, **3)** the use of animal models to develop approaches to reduce the impact of prenatal alcohol exposure.

THE USE OF ANIMAL MODELS TO ADDRESS BASIC QUESTIONS ABOUT ALCOHOL EXPOSURE DURING DEVELOPMENT

While many studies have already addressed how alcohol causes neurodevelopmental damage, questions remain. For example, why the apparent differences in individual susceptibility to prenatal alcohol exposure? Are there key times during development for exposure to cause injury that help explain the extremely heterogeneous and relatively poorly predictable phenotype? What is the role of social factors in FASD? Is there a “safe” exposure level? Does embryonic/fetal tolerance, withdrawal or dependence play a role in the developmental injuries? What are the alcohol sensitive brain structures that are responsible for the social and other complex behavioral abnormalities? These and other complicated questions may best be addressed using translational models (animal models that are very like humans).

On the other hand, model systems that are very dissimilar to humans can offer unique advantages that allow for answering mechanistic questions that cannot be addressed in humans or in the translational animal models. For example, zebra fish larva are transparent and develop external to the mother allowing for visual study of the effects of alcohol on development (Tanguay and Riemers, 2008). A simple nervous system, short generation interval, lack of a placenta and the ability to address basic questions of development and genetics are all advantages of simple non-mammalian animal model systems such as the zebra fish, roundworm, fruit fly, and frog. Avian models also offer several advantages for developmental research. Fertile eggs are inexpensive, commercially available, and require only an incubator to develop. The shell is easily windowed to directly view or manipulate the embryo and easily resealed to continue development. Significant genetic and molecular resources are available and developmental processes are strongly conserved between avian and mammalian embryos (Smith, 2008). While the use of lower order animal models offers opportunities to answer mechanistic questions and to test treatment approaches, verification of these findings in a more human like or translational animal model will be important in many cases.

More phylogenetically advanced animals are required for the study of behavior, learning and in cases where longer developmental periods must be studied. Rodents offer advantages that make them important models of FASD. More recently, it has been recognized that the effects of alcohol on social interactions may play an important role in FASD and may at least partially address why there is so much heterogeneity in the FASD phenotype both observationally and experimentally (reviewed by Kelly et al., 2009). Rodents offer advantages in this area of study because of their short generation interval, the ability to cross-foster and the maternal-infant interactions in rats that effectively model some human

behaviors. The sheep model offers the advantage of a long gestation, an experimental requirement for exploring repeated withdrawal during gestation as a mechanism of damage. Nonhuman primates exhibit more complex social relationships and cognitive functions and share a high degree of gene homology with humans, thus making them a good bridge between studies in other animal models, such as the rodent, and humans. For example, studies have been done using nonhuman primates in the areas of cognitive function, alcohol preference and dopamine system function since they are so similar to humans (Schneider et al., 1997, 2001, 2005, Roberts et al., 2004, Kraemer et al., 2008). They also have the advantages that gestation and early development are similar to the human and their shorter generation interval allows longitudinal studies to be conducted (Schneider et al., 2002). These examples illustrate how the choice of animal model must be made based on the scientific question to be studied.

THE USE OF ANIMAL MODELS TO IMPROVE THE IDENTIFICATION OF AFFECTED INDIVIDUALS

Early identification of FASD can result in children receiving interventions, services and, as a consequence, improved outcomes (Streissguth et al., 1997). Therefore, early identification of children with prenatal alcohol exposure is an important goal of FASD research. Identification of FASD early in a child's life is currently exceedingly difficult and rare; the current means are inadequate. Women who abuse alcohol during pregnancy rarely volunteer this information to healthcare providers. The search for better ways to identify affected individuals currently has three areas of focus. First is the identification of structural abnormalities to identify affected individuals that can be further divided into the use of facial dysmorphology and brain imaging. The second area is the identification of functional abnormalities. The third area is the identification of biomarkers different from the above methodologies.

Facial and Brain Measurements/Imaging

Prenatal alcohol exposure can result in alterations in development of the face or facial dysmorphology and accumulating experimental evidence suggests that changes in the face as a result of prenatal alcohol exposure reflect changes in brain structure and thus serve as an outward sentinel of brain damage (Sulik and Johnson, 1982, Sulik, 2005). Facial dysmorphology is an important screening tool for the identification of children affected by prenatal alcohol exposure. No other screening tool has as yet been proved to be as specific for the identification of affected children. Currently, a diagnosis of FAS requires the identification of facial dymorphology by a highly trained dysmorphologist. The identification of facial dysmorphology is problematic at present because there are very few who are trained to make the identification and only a fraction of children prenatally exposed to alcohol express facial dysmorphology, at least that is currently recognizable. Efforts are underway to create camera and computer systems to detect facial dysmorphology in children (Meintjes et al., 2002). A three-dimensional (3D) facial laser has been used to scan images from children with fetal alcohol syndrome (FAS) to develop an automated diagnostic technique to identify individuals prenatally exposed to alcohol (Fang et al., 2008). The automation of the detection of facial dysmorphology would also make screening for FAS more widely available than is currently possible with the current paucity of trained dysmorphologists. However, efforts to improve the sensitivity of facial dymorphology screening tools may benefit from the use of animal models because human subjects can only be studied retrospectively while animal subjects can be studied prospectively providing for the possibility of identifying changes in the face caused by lower but known doses of alcohol that are more subtle than are currently recognizable by dymorphologists. This work

is currently being pursued in rodent and sheep models (Anthony et al., 2010, Goodlett et al., 2010, Parnell et al., 2006).

Prenatal alcohol exposure can result in alterations in the structural development of the brain (Bookheimer and Sowell, 2005, Riley et al., 2004). Recent advances in neuroimaging with the improvement of speed and resolution of MRI and fMRI, have made it possible to identify structural and functional changes in children with FASD (Spadoni, et al., 2007). The use of animal models may make it possible to improve the sensitivity and specificity of neuroimaging techniques to identify FASD; animal studies allow for control over timing and dose and allow for anatomical conformation of imaging findings. Magnetic resonance microscopy (MRM), magnetic resonance imaging (MRI) at a microscopic level of resolution of brain structures, is being used in the mouse model and provides unprecedented opportunities to define the full spectrum of alcohol's insult to the developing brain (Parnell et al., 2009). This information will be important in confirming and extending human clinical observations. As is the case with facial dysmorphology, only with animal models can prospective studies be performed to explore structural differences currently below our current threshold to appreciate. Current efforts to develop brain imaging screening tools using human subjects are limited because alcohol exposure dose and pattern are not known, potential confounds of nutrition, other substances of abuse and other environmental factors, the inability to utilize very high magnetic field strengths and the inability to corroborate findings using anatomical methods.

Functional Measures of Brain Dysfunction

Children prenatally exposed to alcohol with and without the physical features of FAS demonstrate qualitatively similar deficits in neurobehavior including impairments in memory, attention, reaction time, visuospatial abilities, fine and gross motor skills, social and adaptive functioning, abnormal activity, reactivity, and hyperactivity, attention deficits, lack of inhibition, impaired learning, reduced habituation, feeding difficulties, gait abnormalities, developmental delays, impaired motor skills, hearing abnormalities, and poor state regulation (sleep, jitteriness, and arousal abnormalities). Neurobehavioral deficits identified in children with FASD have been documented in animal studies utilizing a variety of models (for review, see Driscoll, et al., 1990, Kelly et al., 2000). Animal models have been used to identify structural damage to specific brain regions and the neural pathways that are responsible for many of these functional/behavioral deficits. This knowledge is now being exploited to develop screening tools for prenatal alcohol exposure injury by testing for specific functional deficits. Pavlovian conditioning requires a complete neural circuit that includes cerebellar Purkinje cells. Investigations have determined, using animal models, that the rate of acquisition of Pavlovian conditioning is diminished proportionate to cerebellar Purkinje cell loss (Tran et al., 2007, Brown et al., 2008). Cerebellar Purkinje cells are exquisitely sensitive to prenatal alcohol exposure. This technique is valuable because it can be used at different stages of development and it can be used in both animals and humans including very young children. An additional example of how discoveries in animal models have led to the development of functional testing in children with FASD is provided by Savage and coworkers development of a virtual Morris water task test for measuring deficits in spatial navigation (Hamilton et al., 2003). Animal models were utilized first to demonstrate that the hippocampus is important for spatial navigation, second for the development of the Morris water task to measure spatial navigation in animals, third for the demonstration that loss of hippocampal function alters spatial navigation learning and finally the establishment that alcohol exposure during brain development causes hippocampal damage and impairment of spatial navigation as measured by Morris water task (Gianoulakis, 1990, Goodlett and Peterson, 1995, Morris et al., 1982, Squire, 1992, Sutherland et al., 2000). It is expected that animal models will continue to play an important

role in the development of additional functional testing as in the case of the development of the virtual Morris water task, by providing the basic information on the anatomical basis of functional deficiencies and the development of learning measures that specifically measure the particular learning in question. A final important point is that functional testing may identify the more important functional abnormalities that could be present in the absence of structural deficits.

Biomarkers

Because of the difficulty in identifying affected individuals, efforts are underway to identify biological substances in fluids or tissues whose expression changes in response to prenatal alcohol exposure or neurodevelopmental injury associated with prenatal alcohol exposure, biomarkers. While finding effective biomarkers solely by studying human subjects may prove to be successful, using animal models poses the advantage of allowing the collection of a greater range of tissues and fluids that are sometimes necessary in the discovery stage of developing a practical screening tool. Additionally, experiments utilizing animal models will provide known dose, timing relative to development and pattern of alcohol exposure and can allow for the elimination of confounds (subjects using other substances of abuse etc.). One approach being investigated is proteomic screening, the identification of a specific pattern of expressed proteins that is sensitive and specific to alcohol exposure. Support for this approach is based on evidence that prenatal alcohol exposure causes a specific characteristic change in the protein profile in amniotic fluid in mice (Datta et al., 2008). A second approach is to identify specific microRNA whose expression changes (up or down) in response to prenatal alcohol exposure (Miranda et al., 2010). MicroRNA are a recently identified family of non-protein coding RNAs and that can survive in circulation and that have been found to serve as biomarkers for specific cancers (Ng et al., 2009). Efforts are underway to identify microRNA biomarkers in the sheep model of FASD (Balaraman et al., 2010). The sheep model is useful because it can be used to effectively model prenatal alcohol exposure patterns seen in women who abuse alcohol during pregnancy; sheep have a long gestation allowing for all drinking patterns and gestational exposures and all of the equivalent of human prenatal brain development occurs prenatally in sheep. A third approach is the identification of fatty acid ethyl esters (FAEE), stable non-oxidative metabolites of alcohol metabolism that have been detected in mice, guinea pigs, rats and sheep prenatally exposed to alcohol (Littner et al., 2008, Bearer et al., 1996, Brien et al., 2006, Caprara et al., 2005, Mac et al., 1994, Laposata et al., 2000, Soderberg et al., 1999, Kulaga et al., 2006). FAEEs produced during gestation accumulate in hair and meconium in humans as well and studies suggest that they may serve as an effective index of prenatal alcohol exposure (Bearer et al., 1999, 2003, 2005, Chan et al., 2003, Klein et al., 1999.). While animal models will likely serve an important role in the identification of new candidate biomarkers and for their validation, the possibility exists that ideal biomarkers may be expressed only in humans. While this is possible, animal model work in this area may be instrumental in the proof of concept stage of biomarker development.

THE USE OF ANIMAL MODELS TO DEVELOP APPROACHES TO REDUCE THE IMPACT OF PRENATAL ALCOHOL EXPOSURE

Perhaps the most indispensible role for animal models is in the discovery and development of approaches to reduce the impact of prenatal alcohol exposure. There are currently three categories of approaches to reduce the impact of prenatal alcohol exposure: 1) altering, enhancing or enriching the social, learning, sensory or motor environment, 2) nutritional/ nutriceutical interventions, 3) pharmacological interventions.

With the increased emphasis on finding treatment strategies for FASD, attention is being given to the investigation of how the social aspects of postnatal experiences, environmental enrichment and voluntary exercise may impact the outcome in animal models of FASD. Findings from rodent studies (reviewed by Kelly et al., 2009) illustrate the potential for the manipulation of the postnatal social environment to ameliorate or exacerbate perinatal alcohol-induced deficits, including possible trans-generational effects. The simpler social behavior and generation interval of rodents make it a useful model for use in determining how social context impacts the effects of alcohol exposure during development (Kelly et al., 2009). Developing a better understanding of the specific effects from varying social interactions and from environmental enrichment may have important implications for treatment of children with FASD who are consistently characterized as having poor social skills (O'Connor et al., 2006, Schonfield et al., 2009, Kelly et al., 2009).

The promotion of neuroplasticity as a treatment for neurodevelopmental damage caused by prenatal alcohol exposure has shown great promise. Experiments using the rat model have demonstrated the potential therapeutic value of motor training intervention programs that can be applied to human offspring suffering from alcohol-related neurodevelopmental disorder. Complex motor skill training in adult rats showed stimulation of synaptogenesis in the cerebellum, and that Purkinje neurons that survive an early postnatal alcohol insult are capable of substantial experience-induced plasticity (Klintsova, et al., 2002).

In addition to studies using rodents, a ferret model has been developed in which alcohol exposure during a brief period of development impairs ocular dominance plasticity at a later age. This model provides a novel approach to investigate the consequences of fetal alcohol exposure and should contribute to our understanding of how alcohol disrupts neural plasticity (Medina et al., 2003). Animal studies such as these will provide a deeper understanding of the mechanisms by which prenatal alcohol exposure can disrupt the neurochemical and physiological mechanisms of synaptic plasticity that underlie cognition and learning. Information gained may lead to the development of strategies to prevent lifelong cognitive and behavioral handicaps as a result of prenatal alcohol exposure (Savage,et al., 2002). The ferret model also offers a tremendous opportunity to test pharmacology treatment strategies that promote neuroplasticity (Medina et al., 2006.)

A number of promising approaches to achieve neuroprotection are now under investigation, including the use of retinoids, antioxidants, neuromodulatory compounds and nutritional/ nutriceutical interventions. There is evidence that prenatal alcohol exposure disrupts retinoic acid synthesis leading to neurodevelopmental injury prompting studies to determine if retinoid acid supplementation is protective (Shean and Duester, 1993). Recent research has focused on oxidative stress as a potential mechanism for alcohol induced neurodevelopmental damage. While some rodent studies have demonstrated a decrease in ethanol induced reactive oxygen species generation in response to antioxidant therapy (Dong et al., 2008), other studies using structural measures such as Purkinje cell number in the rat cerebellum (Grisel and Chen, 2005) or functional measures such as eye blink classical conditioning in rats have not demonstrated a protective effect. The effectiveness of this approach remains unproven but these studies demonstrate the strength of using multiple measures to evaluate effectiveness when testing therapeutic interventions in an animal model. The neurotrophic peptides SAL and NAP have been shown to prevent the neural tube deficits and the disruption of the genesis and development of serotonin (5-HT) neurons in the raphe nuclei in mice when they were given in conjunction with alcohol (Zhou, et al., 2008). Evidence suggests that alcohol may cause neurodevelopmental injury by altering the availability of, or by increasing the requirements for specific nutrients. Choline supplementation in the rat model administered either prenatally or perinatally has been shown to mitigate the effects of alcohol exposure in rats (Thomas, 2007, 2009).

Disturbances in maternal amino acid levels as a result of alcohol exposure have been demonstrated in pregnant sheep suggesting that alcohol mediated amino acid deficiencies may play a role in mediating neurodevelopmental damage (Ramadoss, et al., 2008). The identification of nutritional protection strategies, their validation and the establishment of safety will require the use of animal models.

SUMMARY

Neurodevelopmental damage as a result of prenatal alcohol exposure is a significant health problem and economic burden in the United States. Animal models have been utilized to make many of the scientific advances in the FAS/FASD field including the validation that alcohol is a teratogen, the identification of the specific sites of injury and the mechanisms of action. Animal models also have provided for the identification of previously unknown teratogenic effects of alcohol that were then identified in humans (Kelly et al., 2009); examples include renal anomalies (DeBeukelaer et al., 1977), auditory deficits (Church, 1987), spatial learning dysfunction (Blanchard et al., 1987) and impairment of eyeblink conditioning (Stanton and Goodlett, 1998).

Because educational efforts have not been successful in reducing the incidence of FASD, there has been increased interest in the development of more sensitive means of identifying affected individuals and the development of protective and treatment strategies. These efforts also will depend on the use of animal models to identify novel approaches, to validate efficacy and to demonstrate safety. No animal develops in the same way as humans or demonstrates the intelligence and complexity of human behavior, but specific animal models accurately model certain aspects of human development and particular human behaviors. The correct choice of animal model involves matching the advantages of a particular animal model to the specific experimental requirements that must be met to answer a specific question. In conclusion, animal models will continue to be invaluable for improving the identification of FASD, the development of protective and treatment strategies and for providing a greater understanding of FASD.

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