LETTERS TO THE EDITOR

A Transition in Fetal Alcohol Syndrome Research: The Shift from Animal Modeling to Human Intervention

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BACKGROUND

Fetal alcohol spectrum disorders (FASD) are a group of conditions that can occur in the offspring of a woman who consumed alcohol during pregnancy (CDC, 2014a,b). FASD refers to a range of disorders, with fetal alcohol syndrome representing the most severe end of the spectrum, which may include symptoms such as: abnormal facial features, small head size, poor coordination, hyperactivity, difficulty paying attention, poor memory, speech/language delays and learning disabilities, intellectual disabilities, low IQ, vision and hearing problems, heart, kidney, and bone problems (Paintner et al., 2012). Diagnosis is often difficult, as there are multiple diagnostic criteria for FASD (Paintner et al., 2012). There is no specific or highly effective treatment, but early intervention can improve a child’s development. Treatment includes medication to manage symptoms (for example, attention deficit and hyperactivity), behavior and education therapy, and parent training (Paintner et al., 2012; Pruett et al., 2013). Because the needs of the affected individual are likely to change as they age, treatment of FASD requires close monitoring and adaptations to the treatment plan (Pruett et al., 2013).

Current estimates of fetal alcohol syndrome frequency in the USA range from 0.2 to 1.5 cases per 1000 live births, according to the Centers for Disease Control and Prevention. Experts believe FASD likely occur three times as frequently as fetal alcohol syndrome (CDC, 2014a,b). Approximately half of all pregnancies in the USA are unplanned, with the result that many women who drink alcohol continue to drink during the early weeks of pregnancy because they do not realize that they are pregnant (Floyd et al., 1999; Finer and Henshaw, 2006). Only about 40% of women realize that they are pregnant by 4 weeks of gestation, a critical period for organ development (Floyd et al., 1999). Between 2006 and 2010, 51.5% of non-pregnant women reported drinking any alcohol in the past 30 days, and 15% reported binge drinking (at least four drinks on one occasion for women). Among pregnant women, 7.6% reported consuming any amount of alcohol, and 1.4% reported binge drinking (CDC, 2014a,b). There is no documented safe amount of alcohol to consume during pregnancy, and there is no time during pregnancy when it is safe to drink (CDC, 2014a,b).

PREVENTION AND INTERVENTION

Despite warnings from surgeons general and educational campaigns targeting women of reproductive age, alcohol-exposed pregnancies continue to occur (Warren et al., 2011). Therefore, a new sociocultural approach is needed not only to convey this message effectively to women of childbearing age, but also to educate women—and men—in general. Many researchers are currently exploring various methods to address this issue.

Prevention

Addressing the high rate of unplanned pregnancy in the USA is an essential step in preventing FASD. One arm of this strategy is ensuring that all women have access to free or low-cost contraception (Waterman et al., 2013). The Affordable Care Act took a step in the right direction by requiring insurance companies to provide contraception as part of women’s preventive health care without patient cost sharing (Waterman et al., 2013). Additionally, it is essential that women of reproductive age be educated on the importance of contraception and preventing unplanned pregnancies, especially if they engage in risky or frequent drinking behavior (Waterman et al., 2013). This can be accomplished through a combination of public health education campaigns and through patient–provider interactions. Access to low-cost contraception, education, and quality health care providers are essential to reducing the burden of unplanned and alcohol-exposed pregnancies.

A number of scientists are attempting to develop tools for reducing alcohol consumption among women of childbearing age. A 2011 study tested the efficacy of a web-based assessment and intervention tool among women of childbearing age receiving Women, Infant and Children (WIC) services. Participants received either personalized feedback or general health information regarding the health effects of alcohol and FASD. The primary outcome measure was a reduction in the number of risky drinking occasions. While there were no significant differences between the two groups, over 70% of participants reported a reduction in risky drinking behavior (Delrahim-Howlett et al., 2011). Similarly, the Healthy Choices campaign compared telephone and in-person brief interventions in women of childbearing age who engaged in risky drinking and did not use effective contraception. The primary outcome measures were decrease in risky drinking behavior and increase in contraception use. There was no significant difference between telephone and in-person interventions, but a significant overall reduction in alcohol consumption and increase in contraceptive use was reported (Wilton et al., 2013).

Despite the promising results of these and other studies, there remains the challenge of harnessing results of scientific studies and applying them in medical practice. A 2013 review article in the Obstetrical and Gynecological Survey summarizes the role of provider knowledge and attitude as a barrier to FASD prevention (Waterman et al., 2013). Results from a 2000 survey revealed only 33% of providers felt adequately prepared to address alcohol use in pregnant patients, while a quarter of respondents felt that medical school training had not adequately prepared them to counsel...
patients about alcohol use during pregnancy (Diekman et al., 2000). Moreover, only half of the providers surveyed counsel all relevant patients about the effects of alcohol use during pregnancy (Diekman et al., 2000). Many health care providers do not agree on acceptable levels of alcohol consumption during pregnancy. While many physicians counsel patients to abstain altogether, others feel 'moderate' alcohol consumption during pregnancy is acceptable (Nevin et al., 2002; Tough et al., 2008; Waterman et al., 2013). A fundamental change in provider performance is needed, which would be facilitated by adequate education of medical and nursing students and training of resident physicians, and by continuing medical education programs addressing the counseling of patients about alcohol use.

Intervention

While an ideal approach to eliminating FASD involves preventing alcohol-exposed pregnancies, improved strategies for providing care for affected individuals must also be developed. Numerous studies have suggested that early intervention improves prognosis and quality of life for individuals with FASD, largely by preventing secondary disabilities (Wilton and Plane, 2006; Paintner et al., 2012; Pruett et al., 2013; CDC, 2014a,b). Currently, many diagnoses depend on paternal report or presence of classical FASD features, such as dysmorphic facial features (Warren et al., 2011; Paintner et al., 2012). Improving diagnostic guidelines and techniques will enable clinicians to make earlier diagnoses and refer patients for intervention services as early as possible.

Neonatal biomarkers of maternal alcohol use, such as fatty acid ethyl esters in meconium, would allow healthcare providers to identify newborns at risk for FASD (Paintner et al., 2012; Memo et al., 2013). As these children grow, diagnostic follow-up visits with physicians would allow for intervention at the earliest sign of disability. Prenatal ultrasound studies seek to determine whether alcohol-exposed fetuses exhibit growth defects that can be detected in utero. 3D laser facial scans use computer algorithms to detect differences in facial features related to prenatal alcohol exposure that may not be detectable to the naked eye (Fang et al., 2008; Mutsangwa et al., 2009). Validation of these techniques, as well as determining how best to provide them in a cost-effective and ethical manner, would enhance health care providers' ability to determine which children and families may need early intervention and support services.

Finally, ensuring that all families have access to quality intervention services for FASD-affected children is essential in preventing secondary disabilities for those children. For example, therapy services can help children learn important skills such as speech and socialization (CDC, 2014a,b). Early diagnosis can also ensure school-age children receive appropriate special education services, tutoring and support in school (Wilton and Plane, 2006; Paintner et al., 2012; Pruett et al., 2013; CDC, 2014a,b). The Family Empowerment Network provides a model for supporting families struggling to find services for children affected by FASD. This network allows providers to refer families for specific services, and also matches families with each other for mentoring and support purposes (Wilton and Plane, 2006). As stable, nonviolent home environments are extremely important in ensuring the success of a child with FASD, future investment in similar networks can help ensure affected families have access to the multiple support modalities they will require.

DECIPHERING DISEASE MECHANISMS

In addition to the prevention, diagnosis and intervention research seeking to prevent and ameliorate the effects of FASD in human patients, a large portion of the FASD research effort is dedicated to mechanistic studies, primarily using animal models with animals ranging from fruit flies and worms to sheep and nonhuman primates. Following the observation of what is now known as FASD in children in the 1960s, animal models were used to confirm that prenatal alcohol exposure caused these defects (Wilson and Cudd, 2011). Other FASD-related abnormalities, such as renal dysfunction and impairment of eyelink conditioning, were identified in animal models as well (DeBeukelaer et al., 1977; Church, 1987). Today, most basic FASD research aims to understand the teratogenic effects of alcohol on the developing fetus, particularly in hopes of developing a therapeutic agent that may reverse damage or prevent further damage. While the goal of such research is certainly desirable, there are a number of biochemical, physiological and environmental factors that severely hamper the translational potential of animal-based FASD research.

Non-mammalian models

The simplest animal models of FASD are non-mammalian species such as zebra fish, roundworms and fruit flies. These species have simple nervous systems and short generation times, and are often used to address basic developmental and genetic questions (Cudd, 2005). Chick embryos are also used for their short generation times and ease of access to the developing embryo. However, in all these cases the absence of a placenta connecting mother and developing embryo represents a significant deviation from mammalian development and is a significant limitation of the systems’ translational relevance to humans. Moreover, some non-mammalian models require large doses of alcohol in order to induce developmental defects, raising concerns about the relevance to human disease (Cudd, 2005).

Mammalian models

Both mice and rats are frequently used in FASD studies. For both species, there is a very large body of literature regarding normal physiology and physical and behavioral development (Cudd, 2005). Because rats have been so well-characterized in behavioral studies, they are frequently utilized to study the effect of alcohol exposure on learning and memory (Brown et al., 2007). Mice have been used to study the effect of alcohol exposure on the development of dysmorphic facial features (Sulik, 2005). Moreover, mice are frequently used by researchers attempting to determine the roles of specific genes in various aspects of FASD (Kleiber et al., 2014).

While transgenic animals are widely used in many fields of research, there are a number of factors that complicate the translational value of data gained from their use. There is no way to control where a transgene is inserted, how many copies are inserted, or how the inserted gene will be translated and processed. The tissue in which a gene is expressed, as well as the timing and abundance of gene expression, all
The hypothalamus

NHP has been linked to changes in the hypothalamic

when interpreting data collected from these animals (Cudd, 2005). As a result, many FASD studies utilizing rodent models are actually performed with newborn animals—removing both the mother and the placenta from consideration.

Another species that has been studied for decades is the sheep. Because they form a large maternal-fetal unit and are easily implanted with instruments, sheep have long been used to study fetal development and physiology. As a result, this species has been extensively utilized to study the effect of alcohol exposure on fetal development (Brien et al., 1985; Gleason and Hotchkiss, 1992). However, surgical implantation of catheters and other instruments requires general anesthesia, which is known to supplement the effects of alcohol (Cudd, 2005). While sheep have a much longer gestation period than rodents or non-mammalian species, brain development occurs earlier in gestation than it does in humans, and a newborn lamb’s brain is 53% of its adult weight (Dobbing and Sands, 1979).

Nonhuman primates (NHP) are most closely related to humans and exhibit many similar behaviors, and they have frequently been used in biomedical research as a result. Like humans, NHP have long gestation periods (Schneider et al., 2002). Moreover, pregnant NHP can be implanted with catheters for monitoring during experimental procedures (Cudd, 2005). As with sheep, however, interactions between anesthetics and alcohol may confound interpretation of results. Moreover, high fetal loss rates have been reported in NHP models of FASD (Cudd, 2005). Sample collection from NHP commonly results in overt fear responses, such as vocalizations and physical resistance (Reinhardt, 2003; Balcombe et al., 2004; Cudd, 2005). A 2005 review of animal models of FASD cited restraint stress in NHP as a confounding issue (Cudd, 2005). Finally, as with all the other species discussed here, the velocity of brain growth differs between NHP and humans. For example, a rhesus macaque is born with 76% of its adult brain weight (Dobbing and Sands, 1979).

It is important to note that alcohol abuse is a uniquely human condition—no other species willingly consumes alcohol. Animals used in alcohol-related experiments are sometimes trained to drink sweetened alcohol, but are frequently injected or gavaged with extremely high doses of ethanol (Aston and Stolman, 1966; Spirduso et al., 1989; Silva et al., 2002). Oral gavage itself has been shown to cause changes in body temperature, hormone levels, and liver function in mice and rats (Germann and Ockert, 1994; Roberts et al., 1995; Brown et al., 2000). Consumption of alcohol in rats has been linked to changes in the hypothalamic–pituitary–adrenal (HPA) axis, which plays a central role in stress responses (Silva et al., 2002, 2009). HPA axis activation and increased corticosteroid production has been reported in NHP following ethanol administration as well (Porcu et al., 2006). Thus researchers are studying the effects of alcohol consumption in species that do not consume ethanol, that metabolize ethanol differently, that receive ethanol via different routes than humans, that have different genetic and physiological environments, and that exhibit stress responses due to housing conditions, frequent handling and alcohol consumption itself (Zorzano and Herrera, 1990). These models are remarkably far removed from alcohol abuse as it occurs in humans.

The National Institute of Alcohol Abuse and Alcoholism (NIAAA) dedicates approximately $30 million annually to fetal alcohol syndrome research (NIAAA, 2014). Based on a search of the NIH’s RePORTER database, approximately $17 million of this funding was allocated for research involving animal models of FASD in 2013 alone (http://projectreporter.nih.gov/reporter.cfm). However, a search of the literature reveals that despite over 4000 FASD-related publications between 1970 and 2014, only 72 clinical studies involving FASD were published (Fig. 1). Only two of these studies tested a therapeutic measure for affected children. One trial tested the effect of methylphenidate in reducing attention deficit symptoms in a group of only four children with FASD. The second study was a phase I trial examining the safety of choline supplementation in children with FASD. No efficacy studies have been reported. Such a small return on an enormous investment of time and financial resources casts doubt on the ability of these models to yield results that can be effectively translated into treatments for FASD-affected individuals.

CONCLUSIONS AND FUTURE PERSPECTIVES

Fetal alcohol syndrome remains the leading cause of preventable birth defects and developmental disorders in the USA. NIAAA provides approximately $30 million annually to FASD-related research (NIAAA, 2014). More than half of this funding is dedicated to basic science research in animal models of FASD. Despite these investments, rates of maternal drinking remain relatively consistent, and no novel therapies have been identified. Given that alcohol abuse is a

Fig. 1. FASD-related publications cited in PubMed since 1970. Results of a PubMed search for FASD-related publications since 1970. Of 4327 FASD-related manuscripts, only 72 were categorized as clinical trials. Only two tested a novel therapeutic in children with FASD.
uniquely human condition, a new multifaceted human-centered approach is needed. Rather than continuing to funnel limited research funding into animal-related studies with limited translational capacity, NIAAA and other funding bodies should invest in research aimed at discovering methods for educating women about the effects of fetal alcohol exposure, developing new diagnostic and treatment paradigms, enhancing family support networks, and developing methods for widespread implementation of these measures. Moreover, medical and nursing schools, residency training programs, and continuing medical education programs should build expertise and confidence among physicians and nurses for counseling patients about the effects of alcohol abuse. An emphasis on screening for alcohol abuse issues will allow providers to recognize patients at risk for alcohol-exposed pregnancies. These strategies will increase the likelihood of physicians referring at-risk patients for the care required to prevent alcohol-exposed pregnancies, and they may have the additional benefit of addressing women’s alcohol use in ways that have lifelong benefits for these women as well as their children.

In order for an animal model to yield results relevant to human FASD, it must exhibit functional or structural disorders similar to those that occur in humans in response to developmental alcohol exposure and occur with mechanism of action similar to those in humans (Wilson and Cudd, 2011). However, despite extensive research efforts, no single animal model or combination of models has been developed that exhibits all the diagnostic criteria of FASD as it occurs in humans (Cudd, 2005). Therefore, emphasis should be placed on human-based research methods that will be directly applicable to human patients. FASD research can be transformed using a wide range of currently available human-based in vitro, in vivo and in silico methodologies and by developing novel human-based techniques. Ultimately, only by refocusing our research and education strategies on the human patients affected by FASD, rather than the distraction of animal models of the disorders, will we successfully prevent alcohol-exposed pregnancies and provide adequate care for those already impacted by them.

Conflict of interest statement. None declared.

REFERENCES

Disulfiram and the Zenalyser®: Teaching an Old Dog New Tricks

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Disulfiram is recommended by the National Institute for Health and Care Excellence as a cost effective drug for the treatment of alcohol dependence (NICE, 2011). Nevertheless, there continues to be suspicion regarding the usefulness of this medication, predominantly arising from the perception that compliance is poor. Several attempts to improve compliance have been made, the most notable of those being supervised consumption (e.g. Wright and Moore, 1990; Chick et al., 1992). Supervision is, however, without its limitations—relationships break down, individuals are mobile, supervisors weary of their task.

The German Out-patient Long term Intensive Therapy for Alcoholics (OLITA) programme demonstrated that disulfiram can be extremely effective in maintaining abstinence from alcohol (Krampe et al., 2007). Over 50% of the 180 patients studied remained abstinent from alcohol for 9 years with high frequency support and long-term treatment. However, it is difficult to see how these key ingredients can be incorporated into services that are over-worked, under-resourced, and in locations distant from their patients.

In 2006 the author of this letter, together with colleagues, published the results of a study which demonstrated that the metabolic products of disulfiram can be identified on a sample of breath (Fletcher et al., 2006). The specificity and sensitivity of the technique are 100% if the patient is prescribed 200 mg disulfiram daily. Over subsequent years continuing work has brought this finding to the point of clinical application. By combining disulfiram and breath alcohol measurements in a small hand-held breathalyser and adding appropriate software, a device has now been manufactured that allows individuals prescribed disulfiram to be remotely supported and monitored on a daily basis from any geographical location with internet access.

In brief, this device, a Zenalyser®, works in the following way: a patient blows into the instrument, connects it to a computer and the result is exported to the treating team—a process that takes <45 s from start to finish. The treating team read the result and email the patient back, which takes no more than a couple of minutes depending on the content of the email. Alternatively, the Zenalyser can be kept at the treating base, and patients can attend at frequent intervals to provide breath samples.

Clinical observations (unpublished data) on ten severely alcohol-dependent patients followed up after in-patient detoxification for up to 3 years (mean = 11.3 months), have revealed very high disulfiram compliance rates, which is the main purpose of the technique. Nine out of 10 patients remained totally abstinent from alcohol. One cirrhotic female patient had two brief relapses of 1 week each during an 8-month period of daily support with the Zenalyser—this exceeded, by some considerable margin, her responses to treatment in the previous 30 years, despite three periods of residential rehabilitation.

There have been, however, a number of other benefits observed that were not anticipated and which go beyond improving disulfiram compliance rates. These are as follows:

- Relapse can be anticipated before it occurs, being inferred from rapidly falling disulfiram metabolite levels, the appearance of alcohol on the breath, or because the patient stops sending data
- Out-patient follow-up no longer needs to be routine, but can be reserved for times of high risk thus reducing out-patient activity and other factors, e.g. travel time, carbon footprint
- Daily support and monitoring can be provided together with encouragement, feedback, warnings, etc.—all of the components of good motivational enhancement—very rapidly, cheaply and with minimal staffing requirements
- Patients can be supported and monitored on a daily basis even when they travel to foreign countries
- Patients have been able to use Zenalyser data in legal situations, e.g. alcohol-dependent mothers involved in child protection cases; and doctors under medical supervision from their governing body

Feedback from families and patients shows that they appreciate this technique, being reassured that when a patient leaves...