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Original Article

Computational Models of Ethanol-induced Neurodevelopmental Toxicity Across Species: Implications for Risk Assessment

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Computational, systems-based approaches can provide a quantitative construct for evaluating risk in the context of mechanistic data. Previously, we developed computational models for the rat, mouse, rhesus monkey, and human, describing the acquisition of adult neuron number in the neocortex during the key neurodevelopmental processes of neurogenesis and synaptogenesis. Here we apply mechanistic data from the rat describing ethanol-induced toxicity in the developing neocortex to evaluate the utility of these models for analyzing neurodevelopmental toxicity across species. Our model can explain long-term neocortical neuronal loss in the rodent model after in utero exposure to ethanol based on inhibition of proliferation during neurogenesis. Our human model predicts a significant neuronal deficit after daily peak BECs reaching 10–20 mg/dl, which is the approximate BEC reached after drinking one standard drink within one hour. In contrast, peak daily BECs of 100 mg/dl are necessary to predict similar deficits in the rat. Our model prediction of increased sensitivity of primate species to ethanol-induced inhibition of proliferation is based on application of in vivo experimental data from primates showing a prolonged rapid growth period in the primate versus rodent neuronal progenitor population. To place our predictions into a broader context, we evaluate the evidence for functional low-dose effects across rats, monkeys, and humans. Results from this critical evaluation suggest subtle effects are evident at doses causing peak BECs of approximately 20 mg/dl daily, corroborating our model predictions. Our example highlights the utility of a systems-based modeling approach in risk assessment. *Birth Defects Res (Part B)* 83:1–11, 2008. © 2007 Wiley-Liss, Inc.

Key words: ***cross-species; neocortex; mathematical model; neuron number neurogenesis; evolution; cell cycle; rhesus monkey; apoptosis***

INTRODUCTION

Interspecies extrapolation is a central issue in human health research, as the rodent is frequently utilized for efficacy, toxicity, and mechanistic evaluations in all fields of human health research. In toxicity risk assessments, a 10-fold uncertainty or safety factor is often employed to account for potential interspecies variability in toxicokinetics and for toxicodynamics for non-cancer endpoints (Dourson and Stara, 1983). However, the development of more biologically based methods is an important research goal (Bogdanffy et al., 2001; Renwick, 1993; USEPA, 2003). Allometric principles have been proposed for interspecies extrapolation when no other data are available (Beck and Clewell, 2001), either according to caloric demand or body weight. However, allometric scaling did not predict toxicity well using a set of LD_{50} values from eight species across 217 substances (Schneider et al., 2004).

To explore a mechanistic-based framework for interspecies extrapolations in developmental toxicology, we are using computational models to identify key interspecies differences in normal developmental processes as well as identifying and applying species-specific responses to toxicants (Faustman et al., 1999; Gohlke et al., 2002, 2004, 2005, 2007; Leroux et al., 1996; Lewandowski et al., 2003a). Here we explore an application of this framework using ethanol-induced developmental neurotoxicity as a case study.

Alcohol ingestion during pregnancy may result in one or more detrimental, long-lasting effects termed Fetal Alcohol Spectrum Disorders (FASD), including

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general growth retardation, abnormal brain development, microcephaly, mental retardation, and specific craniofacial malformations, depending on the dose and timing of exposure (Abel and Sokol, 1986; Barr and Streissguth, 2001; Coles, 1992; Streissguth, 1986). Estimates of the prevalence of Fetal Alcohol Syndrome (FAS), the most severe form of FASD, range from 0.5 to 2.0 per 1,000 births in the United States. However, within specific American Indian groups, the prevalence may be as high as 8.97 per 1,000 births (May et al., 2004), and in South Africa full FAS is seen at a staggering rate of 68 to 89.2 per 1,000 births (May et al., 2007). While prevalence of full FAS has been estimated, due to better diagnoses based on the relative severity of the disorder, children born with other FASD conditions may go unnoticed and may account for as many as five times the number of full FAS cases (May and Gossage, 2001; Sampson et al., 1997). Fetal Alcohol Spectrum Disorders are a serious public health problem, and FAS cases alone are estimated to cost as much as 4 billion dollars annually in the United States (Lupton et al., 2004), yet there is still uncertainty regarding the risk associated with low-level exposure to alcohol during pregnancy (Kaskutas and Graves, 2001; May et al., 2004).

It is well recognized that neurodevelopmental effects are a sensitive endpoint after in utero exposure to ethanol (Sampson et al., 2000a; Streissguth et al., 1989b). Deficits in intelligence and cognition, language, mathematical skills, motor function, social behavior, and executive functioning are consistently found in children who were exposed to alcohol in utero (Coles, 1992; Connor et al., 2000; Kelly et al., 2000; Kopera-Frye et al., 1996; Streissguth, 1986). Moreover, dose-related effects are evident for behavioral and cognitive measures such as hyperactivity and IQ (Streissguth, 1986; Streissguth et al., 1998). Deficits in fine motor coordination and learning and memory are thought to primarily reflect ethanol effects on the cerebellum and the hippocampus, respectively, whereas cognitive and behavioral abnormalities, such as hyperactivity, are likely to be more related to neocortical deficits (Hoffman and Tabakoff, 1996; Matthews and Simson, 1998; Mooney et al., 1996). A case-control study of attention-deficit hyperactivity disorder (ADHD) showed cases were 2.5 times more likely to have been exposed to alcohol in utero independently of association with prenatal exposure to nicotine and other drugs (Mick et al., 2002). Learning difficulties, most notably in mathematics and language, are evident even when documentable mental retardation is not, suggesting the hippocampus and neocortex are particularly sensitive areas of the brain (Coles et al., 1991; Kopera-Frye et al., 1996; Shaywitz et al., 1980, 1981).

The developing neocortex is particularly sensitive to ethanol-induced toxicity. When brain surface abnormalities are analyzed, surface area deficits become evident in the frontal lobe as well as the parietal lobe of the neocortex (Riley et al., 2004). These morphological findings are consistent with the behavioral abnormalities seen in children exposed to alcohol in utero linked to the frontal lobe functioning such as response inhibition, behavioral control, and executive functioning (Connor et al., 2000). Furthermore, studies in non-human primates given doses equivalent to 1 drink per day (peak BEC of 20–50 mg/dl) in utero show permanent behavioral deficits specifically in attention span and executive functioning, again suggesting very sensitive functional deficits related to neocortical structures (Roberts et al., 2004; Schneider et al., 2001b). Finally, a critical review of several animal and human studies indicates peak BEC is the most salient dose metric for ethanol-induced neurodevelopmental toxicity (Driscoll et al., 1990).

Previously, we developed a model for normal rat neocortical development and applied ethanol induced effects on both neocortical neuronal proliferation and neuronal cell death (Gohlke et al., 2002, 2005). Our simulations suggest ethanol-induced effects during neurogenesis may cause greater permanent neuronal loss in the adult neocortex after lower doses compared with ethanol-induced neuronal loss attributed to the induction of apoptosis during synaptogenesis (Gohlke et al., 2005). Therefore, we hypothesize ethanol-induced effects on the proliferative stage of neocortical development are particularly detrimental to neocortical functioning. This hypothesis is supported by experiments in rats and non-human primates. For example, significant decreases in rat neocortical neuronal number are seen after exposure to ethanol during neurogenesis, but no decrease in neuronal populations are found after postnatal exposure, when induction of apoptosis is most prevalent (Mooney et al., 1996). In addition, alcohol exposure as it relates to neurobehavioral outcomes in rhesus monkeys is especially sensitive to exposure during earlier, primarily proliferative, neurodevelopmental processes (gestational days 0–50) when compared to later exposure scenarios when normal waves of apoptosis occur (gestational days 50–135) (Schneider et al., 2001a). Therefore, for interspecies comparisons of ethanol-induced developmental neurotoxicity, we have focused on neocortical neurogenesis as a particularly sensitive process (Fig. 1).

In the present analysis, rodent-derived data on ethanol-induced inhibition of the cell cycle are applied to computational models previously developed for rhesus monkey and human neocortical development (Gohlke et al., 2007) to assess our mechanistic-based computational methodology for interspecies extrapolation. We then compare our dose-response predictions to dose-response relationships found in the epidemiological, animal, and in vitro literature. This rich database for ethanol developmental neurotoxicity found in the literature provides an extensive source of behavioral and morphological data to assess hypotheses generated by our computational model predictions.

METHODS

The construct of our computational models for normal rat, monkey, and human neocortical development have been described previously (Gohlke et al., 2002, 2004, 2007). In this stochastic model, the X cell represents a progenitor cell in the ventricular epithelium where it can divide, differentiate into a Y cell, or die according to transition rates of proliferation (λ_1), differentiation (ν), or death (μ_1) (Fig. 1). Early in neurogenesis, X cells are primarily dividing and have a low differentiation rate. However, the differentiation rate progressively increases through neurogenesis as X cells exit the cell cycle, become postmitotic, and begin migration out of the ventricular region to become young neurons in the developing cortical plate. In our current models, X cells

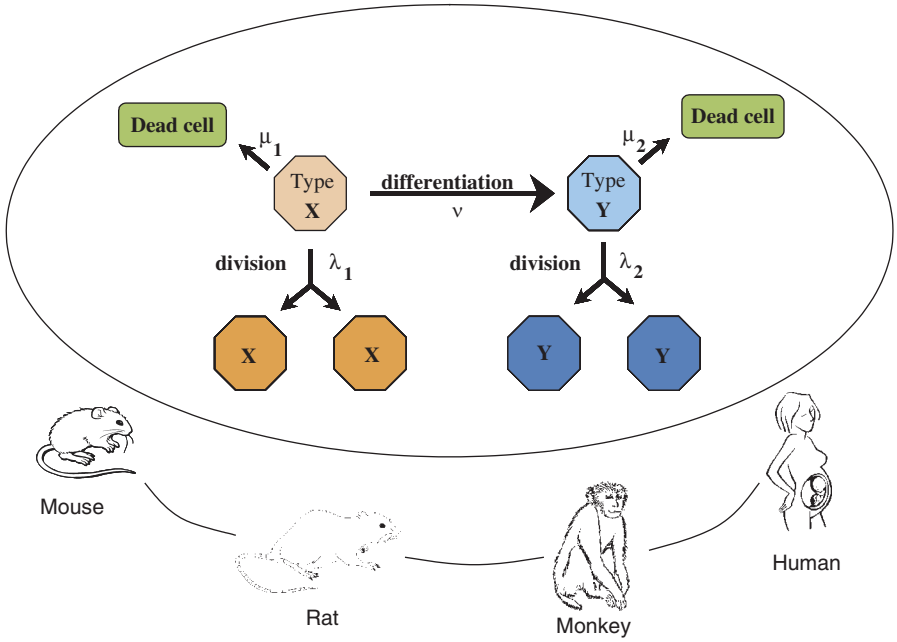

Fig. 1. Framework for cross-species extrapolation of ethanol-induced developmental neocortical toxicity. We have previously developed normal models of neocortical neurogenesis for rat, rhesus monkey, and human (Gohlke et al., 2002, 2007). Here, we apply rodent-derived ethanol-induced inhibition of the cell cycle length during neocortcial neurogenesis to our models for rhesus monkey and human to estimate cross-species toxicodynamic differences. [Color figure can be viewed in the online issue which is available at www.interscience.wiley.com]

have a division rate (λ_1) based on time-dependent species-specific cell cycle length data showing that cell cycle lengths in developing neurons in primate species are considerably longer (ranging between 23 and 54 h vs. 11 and 20 h for rats). Y cells have time-dependent species-specific death rates (μ_2) based on in vivo experimental data, while the division rate is set to zero, as Y cells are defined as postmitotic neurons destined for the cortical plate. A detailed comparison of rodent versus primate neurogenesis has been published previously (Gohlke et al., 2007). All rates are modeled as Poisson processes allowed to vary according to an exponential distribution and are based on numerous in vivo experimental datasets described in the previous publications cited above. The numbers of X and Y cells at time (t) can be derived through a first-order differential equation and can be approximated through the solution matrix described previously (Leroux et al., 1996).

Dose-Response Simulations

In the absence of ethanol-induced perturbation of cell cycle length data in species beyond the rodent, we assume that ethanol-induced lengthening of the cell cycle is consistent across species. The rat-derived data of Miller and Kuhn (1995) were applied to simulate ethanol-induced decreases to the proliferation rates of X cells in the monkey and human models. This dataset has been described previously and was used to simulate ethanol-induced effects in our rat model (Gohlke et al., 2002). Briefly, this dataset shows decreasing effects of ethanol on the proliferation rate over the 7-day period of neurogenesis in the rat. Here, we stretch this rodent dataset of ethanol-induced inhibition during neocortical neurogenesis to fit the 60-day period of monkey neocortical neurogenesis (Kornack, 2000) and 84 days of human neocortical neurogenesis (Chan et al., 2002). These simulations rely on the hypothesis that the developmental timing and peak daily exposure to ethanol during neurogenesis are the salient dose metrics, as has been shown in other studies (Driscoll et al., 1990; Maier and West, 2001). Therefore, since we want to model exposure over the entire neurogenesis period for each species, we are required to assume a greater total dose in the monkey and human.

As in our original rat model (Gohlke et al., 2002), two dose-response relationships were considered. The first is an exponential Weibull dose-response, whereas the second uses a dose-squared function in the original Weibull equation. Therefore, the dose-response simulations can be described by:

$\lambda_1 =$ untreated baseline $*e^{(drc*dose)}$

or:

$\lambda_1 =$ untreated baseline $*e^{(drc*dose\text{^}2)}$

where drc is the time-dependent dose-response coefficient derived from regressing the amount of lengthening of the cell cycle at different doses that best fit the rat in vivo cell cycle length data at 150 mg/dl (Miller and Kuhn, 1995). As the differentiation rate is dependent on the cell cycle length (for further details, see Gohlke et al., 2002), the differentiation rate also changes slightly with dose according to a Weibull function given by:

$\nu =$ untreated baseline $*e^{(-.0026*dose)}$

RESULTS

The general construct of the computational model for developmental processes developed by Leroux et al.

(1996) is shown in Figure 1 to show how it is used to model key neurodevelopmental processes such as proliferation, differentiation, and apoptosis across species. Ethanol-induced changes in neuronal progenitor cell cycle kinetics during the proliferation period of the neocortex have been documented in the rodent. These effects can be simulated in our model by changing the proliferation rate of X cells. Effects seen include a reduction in the proliferating population and increased length of the cell cycle, both contributing to fewer numbers of neurons being generated (Miller, 1989, 1992; Miller and Kuhn, 1995). In these studies, a cumulative BrdU incorporation technique showed moderate alcohol intake (reaching a peak BEC of 153 mg/dl once daily, which is approximately equivalent to imbibing 3–5 drinks over 2 h) significantly increased the cell cycle lengths of the proliferating cells of the dorsal neocortices (Miller and Kuhn, 1995; Nowakowski et al., 1989). More specifically, they showed a 30% increase in cell cycle length (18 h compared with 11 h) during early neocortical neurogenesis (Days 13–16). However, the increase was not constant throughout neurogenesis. As normal neurogenesis proceeds, the cell cycling rate naturally becomes longer, whereas the ethanol-exposed tissues showed the same cell cycling rate throughout cortical neurogenesis.

Model Predictions

Using a Weibull dose-response function, the ethanol-induced inhibition of the proliferation rate of X cells is applied to our monkey and human models (Fig. 2). When compared to our rat model, the simulations suggest that both primate species may be more sensitive to ethanol-induced cell loss due to inhibition of proliferation during neocortical neurogenesis, predicting approximately 30% neuronal loss at peak daily exposures resulting in a BEC of 20 mg/dl in humans versus 100 mg/dl in rats. This result is significant based on confidence intervals using intraspecies variability reported in stereological studies of normal neocortical neuron number in the rat or human (Duffell et al., 2000; Pakkenberg and Gundersen, 1997). When we consider a dose-squared parameter in the original Weibull function for our dose-response relationship, similar cross-species results are seen in that primate species are more sensitive to this inhibition of proliferation, but only between daily exposures causing a peak blood ethanol concentration of approximately 60 and 220 mg/dl (Fig. 3). The positive slope at the initial doses using a dose-squared parameter is due to the concomitant decrease in differentiation rate even as the cell cycle length is increased with dose. Therefore, more cells stay in the progenitor population and ultimately generate more neurons at lower doses when using the dose-square parameter for effects on cell cycle length.

Comparison of Model to Independent In Vivo and In Vitro Datasets

We compare our model predictions to rodent-derived in vivo datasets in which long-term neuronal loss in prefrontal and somatosensory neocortical regions is stereologically estimated after exposure during neocortical neurogenesis (Figs. 2 and 3). Rodent dose response models predict the neuronal loss seen in rat prefrontal cortex. However, the models slightly overestimate the neuronal cell loss when compared to experimental data derived from the rat somatosensory cortex. This suggests differential spatial susceptibility to ethanol-induced

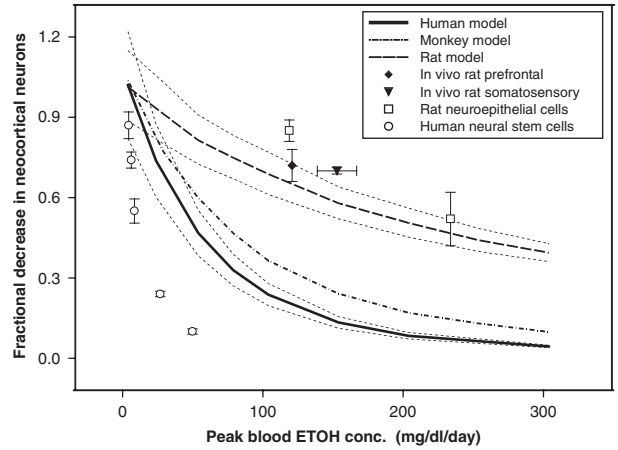

Fig. 2. Cross-species dose-response simulations using a Weibull dose-response function where λ_1 = untreated baseline $*e^{(drp*dose)}$. Human, monkey, and rat dose-response simulations are based on the rat-derived experimental data of Miller and Kuhn (1995). Dotted lines indicate 95% population intervals based on intraspecies variability reported in stereological studies of normal neocortical neuronal number in the rat or human (Duffell et al., 2000; Pakkenberg and Gundersen, 1997). Population intervals for the monkey model are omitted for clarity. For comparison, stereologically determined long-term neocortical neuronal loss in the somatosensory region (down triangle) and the medial prefrontal region (diamond) are shown when exposure occurs during neocortical neurogenesis (Mihalick et al., 2001; Miller and Potempa, 1990). In vitro dose-response relationships are shown for inhibition of BrdU incorporation in primary neuroepithelial cells dissociated from the embryonic rat telencephalon (E13) (open square) (Ma et al., 2003) or cell viability in an undifferentiated human neural stem cell culture (open circle) (Hao et al., 2003). Error bars represent reported SEM.

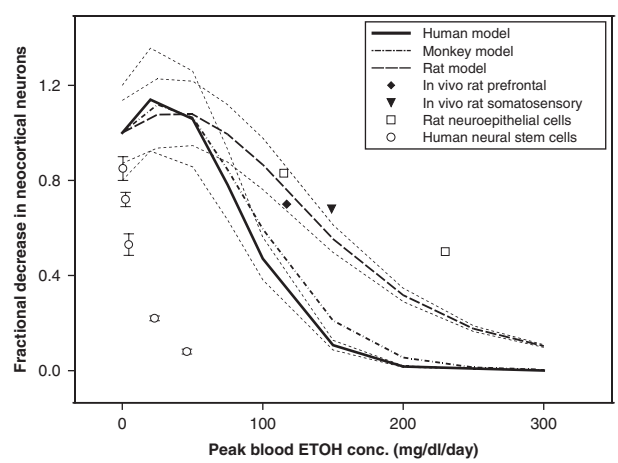

Fig. 3. Cross-species dose-response simulations using the Weibull function with a dose squared parameter defined as λ_1 = untreated baseline $*e^{(drp*dose2)}$. We have also plotted independent stereological studies showing long-term neocortical neuronal loss in the somatosensory region (down triangle) and the medial prefrontal region (diamond) when exposure occurs during neocortical neurogenesis and a rat (open square) and human (open circle) derived in an in vitro study. See Figure 2 legend for further details.

neocortical toxicity may be an important parameter to consider in future modeling endeavors.

We also plot dose-response relationships determined in in vitro systems for comparison with our simulations (Figs. 2 and 3). As a model of ethanol-induced toxicity to rodent neocortical neurogenesis, primary cultures of neuroepithelial cells dissociated from the embryonic rat telencephalon on E13 were exposed to ethanol and ^{3}[H]-thymidine incorporation was measured (Ma et al., 2003). The results of this study compare well with our rodent model predictions, with the Weibull function shown in Figure 2 predicting neuronal loss at the high dose level more accurately than the Weibull dose-squared function illustrated in Figure 3. We compare our primate model predictions to a dose-response relationship from an undifferentiated human neural stem cell culture derived from fetal human brain tissue (11–21 weeks) treated with varying concentrations of ethanol for 12 h and monitored for viability using the MTT assay (Hao et al., 2003). Consistent with our model predictions, the human-derived primary culture system shows increased sensitivity to ethanol-induced damage when compared to findings in the rat primary culture system, showing a significant loss of viability at a very low concentration of ethanol (0.46 mg/dl). However, our primate model results do not predict the level of sensitivity seen in this in vitro culture system where all cells are lost at an ethanol concentration of approximately 50 mg/dl.

Analysis of Independent Studies of Low-Dose Effects Across Species

We have compiled rat, monkey, and human behavioral, biochemical, and morphological studies looking at sensitive neurodevelopmental endpoints of ethanol toxicity to evaluate our model results in the broader context of functional endpoints seen after low-dose ethanol exposure (Fig. 4), enabling us to evaluate our model's utility in risk assessment. Although animal studies have been used extensively to determine ethanol-induced neurodevelopmental effects, only a few have implemented low to moderate exposure paradigms (Table 1). Learning that requires response inhibition and hyperactivity are sensitive behavioral effects seen in rats after low exposure during gestation (Lochry and Riley, 1980; Riley et al., 1979; Savage et al., 2002; Vaglenova and Petkov, 1998). Neurochemical alterations are seen in the serotoninergic system in the striatum and frontal cortex and the glutamate system in the hippocampus (Evrard et al., 2003; Savage et al., 2002). Minor malformations during neural tube midline development are seen at low exposure levels. Furthermore, when the corpus callosum is analyzed in detail, abnormalities are seen in the length and number of dendrites shortly after birth (Qiang et al., 2002).

Studies with non-human primates show moderate ethanol exposure (peak BEC of 20–50 mg/dl comparable to 1 to 2 drinks per day) during pregnancy compromises

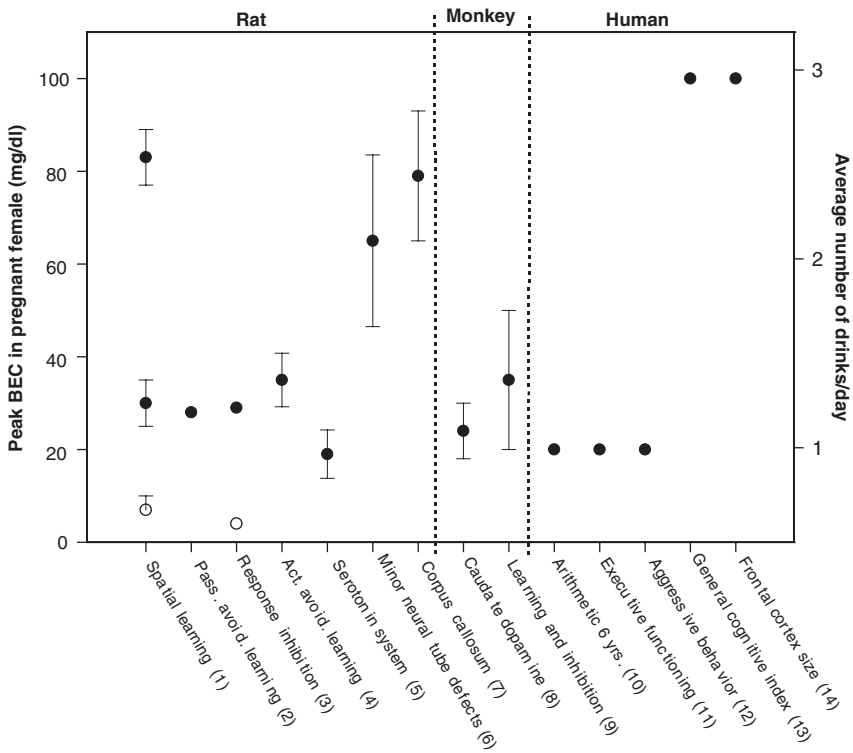

Fig. 4. Ethanol-induced developmental toxicity dose-response comparisons. Human, monkey, and rat behavioral and morphological studies are compared using the peak blood ethanol concentration experimentally estimated in animal studies and average drinks per day in human studies as dose metric. The No Observed Adverse Effect Level (NOAEL) is indicated by an open circle, whereas the Lowest Observed Adverse Effect Level (LOAEL) is indicated by a filled circle. Numbers in parentheses refer to a more detailed description of studies in Table 1. Human BECs are estimated from average drinks per day by assuming 1 drink = 0.5 oz. of absolute alcohol and will produce a peak BEC of 20 mg/dl (Driscoll et al., 1990).

Table 1
Selected Studies of Interspecies Comparisons of Low Dose Effects of Prenatal Alcohol Exposure

Key to figure	Species	Animal exposure paradigm or epidemiology study population	Outcome measure/s	Reference/s
1	Rat	2, 3, and 5% ethanol liquid diets daily throughout gestation	Spatial learning on moving platform Morris Water Task and hippocampal glutamate release at 6 months	(Savage et al., 2002)
2	Rat	17% ethanol liquid diet daily on E6 to E16	Passive avoidance learning at 7 wks	(Lochry and Riley, 1980)
3	Rat	8 and 19% ethanol liquid diet daily on E6 to E16	Response inhibition (hyperactivity) at 7 wks	(Riley et al., 1979)
4	Rat	Intubation of 1 g/kg/day daily throughout pregnancy	Two-way active avoidance acquisition (learning and memory) at 9 wks. and 5 months	(Vaglenova and Petkov, 1998)
5	Rat	6.6% ethanol in drinking water daily through pregnancy	Serotoninergic system in hippocampus, striatum and frontal cortex at P21	(Evrard et al., 2003)
6	Rat	20% ethanol liquid diet daily from E7 to E18	Compromised neural tube midline development, ventricular enlargement at E13, E15 or E18	(Zhou et al., 2003)
7	Rat	Intubation of 1.2 g/kg ethanol daily from E11 to E20	Morphology of corpus callosum including length and number of dendritic branches on G29	(Qiang et al., 2002)
8	Monkey	Intubation of .3, .6, 1.2, 1.8, 2.5, or 4.1 g/kg weekly throughout gestation	Infant neurobehavioral (WGTA), micropthalmia, retinal ganglion cell loss, motor development, stiatal and caudate dopamine at 6 months	(Clarren et al., 1988, 1990)
9	Monkey	Voluntary consumption of .6 g/kg of 6.6% ethanol solution daily from E0-E50, E50-E135, or E0-E165	Infant behavioral assessment scale (IBAS), adolescent learning and behavior with nonmatching-to-sample task (NMS)	(Schneider et al., 2001a,b)
10	Human	Mostly low socioeconomic status, inner city women between 1983 and 1986 in Pittsburgh (N = 595), alcohol use assessed at each trimester of pregnancy and at 8, 18, 36 months, and 6 years postpartum	Neurobehavioral assessment at 3, 6 and 10 yrs. CPT-3 (measures attention span), neuropsychological test battery (Wisconsin, WRAML, Trail Making, Stroop, Grooved pegboard, CPT-2), WRAT-R	(Chandler et al., 1996; Day et al., 1990, 1991a,b, 1994; Day and Richardson, 1991; Geva et al., 1993; Goldschmidt et al., 1996)
11	Human	Mostly Caucasian, married, middle-class and college educated women in Seattle, WA between 1974-1975 (N = 500), alcohol ingestion assessment during the 5th month of pregnancy	Various neurodevelopmental outcomes in infants through 14 yrs. Including but not limited to Apgar, Brazelton, Bayley mental index, IQ at 4 yrs., teacher ratings of undesirable behavior, IQ at 7 yrs., cognitive and vigilance tasks at 14 yrs.	(Barr and Streissguth, 2001; Streissguth, 1986; Streissguth et al., 1989a,b; Streissguth et al., 1981) (Sampson et al., 2000b)
12	Human	Disadvantaged, inner-city, mostly African American women in Detroit, MI between 1986 and 1989 (N = 480), alcohol consumption assessed during each prenatal visit	Children were examined at birth through 7 yrs. and neurodevelopmental endpoints analyzed included Bayley scores, Elicited Play, and Processing Speed, IQ, Achenback Child Behavior Checklist (CBCL)	(Jacobson et al., 1998; Sokol et al., 2001; Sood et al., 2001)
13	Human	Low socioeconomic status women between 1985 and 1986 in Roubaix, France (N = 347), alcohol consumption assessed during 2nd trimester	General Cognitive Index (GCI) and pyschomotor development (McCarthy scales), and minor neurological dysfunctions through age 4.5 years	(Larroque and Kaminski, 1998)
14	Human	Prenatal clinics in Denver between 12 and 42 wks gestation (N = 167), asked about alcohol consumption at conception	Complete brain morphometric study using ultrasonography (12-24 wks.), size of frontal cortex only salient measure	(Wass et al., 2001)

*AA is ounces of absolute alcohol (.5 oz. in a 12 oz. can of beer) and 1.5 AA/day would be approx 100 mg/dl if consumed in one hour (Driscoll et al., 1990).

neurodevelopment (Clarren et al., 1988, 1990; Schneider et al., 2001a,b). Although the monkey population examined by Clarren et al. did not show significant deficits at the lower doses tested, they did note increased frequencies of aberrant scores on neurobehavioral tests as well as morphological abnormalities such as micropthalmia, retinal ganglion cell loss, and increased striatal and caudate dopamine levels. These animals were dosed weekly, producing peaks of approximately 25 mg/dl. It should be noted that the low sample size (n = 6 per dose group) in this analysis may not allow detection of effects at the lower dose levels. Schneider et al. found significant deficits in neurobehavior when pregnant rhesus monkeys were given moderate daily doses of ethanol and, furthermore, showed exposure during early pregnancy (gestational days 1 through 50) is as deleterious to neonatal neurobehavior as late-gestational (gestational days 50–135), or continuous exposure (Schneider et al., 2001a,b).

Four epidemiology studies are analyzed with two of the cohorts (Seattle and Pittsburgh) containing a majority of women reporting low to moderate ethanol intake (Table 1) (average of 1 to 2 drinks/day) during pregnancy (Barr and Streissguth, 2001; Day et al., 1991a; Goldschmidt et al., 2004; Streissguth et al., 1981). The Seattle and Pittsburgh studies describe neurobehavioral effects down to the lowest non-zero exposure (Goldschmidt et al., 1996; Sampson et al., 2000a). The Detroit study indicates effects on aggressive behavior in 7-year-olds down to levels of approximately 1 drink/week (or 0.3 AA/day) when using average ounces of absolute alcohol per day (AA/day) as the dose metric (Sood et al., 2001). Other studies have shown a significant relationship between alcohol exposure in utero and neuropsychiatric disorders or aberrant social behavior later in life (Burd et al., 2003; Kelly et al., 2000). However, it should be noted that although this population had relatively low average levels of drinking, they tended to consume alcohol on average 2 days per week. In contrast, the prospective cohort studied in Roubaix, France, tended to drink daily, but significant effects were not seen in women who ingested less than 3 drinks/day (Larroque and Kaminski, 1998).

The most salient dose metric in animal studies is peak BEC and in epidemiological studies is number of drinks per drinking occasion (Driscoll et al., 1990; Maier and West, 2001). Therefore, we have shown the three U.S. epidemiology studies to have a LOAEL at 20 mg/dl, which would be the expected BEC after drinking one drink. As of yet, none of the epidemiological literature has looked at exposure scenarios in which less than one drink is ingested per drinking occasion, and, in fact, there may not be an adequate population of pregnant women who regularly drink less than 1 drink per drinking occasion to ascertain the effects, if any, at this level. Therefore, we must rely on animal studies to determine if effects are evident below this level.

No Observed Adverse Effect Levels (NOAELs) are described in rat studies (Riley et al., 1979; Savage et al., 2002) looking at behavioral deficits at peak BECs of 4 and 7 mg/dl (Fig. 4). Based on these tenuous data, if a threshold does exist, it suggests it would occur at BECs reached after drinking between 1/4 and 1 drink. However, it should be noted, a precise measurement of peak BECs is difficult in animal studies and becomes even more challenging to estimate in human epidemiology studies relying on questionnaire data. Therefore, the peak BECs shown in Figure 4 should be viewed as approximate ranges of exposure and not precise measurements.

Comparison of Model Results to Functional Studies

The above analysis of independent studies of neurodevelopmental endpoints in rats, monkeys, and humans suggests the original Weibull dose-response function applied in our model (Fig. 2) may more accurately reflect the dose-response relationship suggested by these in vivo studies when compared to the Weibull dose-squared response function, which predicts no significant effects at peak BECs below approximately 60 mg/dl (Fig. 3). Because the coefficient of variation of normal neocortical neuronal number in humans is approximately 20% (Pakkenberg and Gundersen, 1997), we can define an adverse effect level at neocortical neuronal loss of 20% or greater in humans. Using this definition of adverse effect, our original Weibull dose-response relationship model predicts a significant neuronal cell loss at peak BECs between 10 and 20 mg/dl, whereas the second model with a dose-squared parameter predicts significant loss between peak BECs of 60–75 mg/dl.

DISCUSSION

We have developed computational approaches to address ethanol-induced developmental neurotoxicity in three species, namely the rat, monkey, and human, using the general framework for biologically based dose-response modeling for developmental toxicology proposed by Leroux et al. (1996) (Fig. 1). Our current model suggests primate species may be more sensitive to reductions in neocortical neuronal number when rat-derived data of ethanol-induced inhibition of neurogenesis are applied to our rhesus monkey and human models. This result is corroborated by an analysis of in vitro culture systems suggesting human-derived neural progenitor cells are more sensitive to ethanol-induced toxicity than neural progenitor cells derived from the rat. Finally, a comparison of functional toxicity endpoints across rat, monkey, and human suggests a lowest observed adverse effect level at a peak blood ethanol concentration of approximately 20 mg/dl in humans. This analysis suggests the Weibull dose response function is the most appropriate in our current model construct for prediction of ethanol-induced developmental neurotoxicity in the human.

Model Predictions of Increased Sensitivity in Primates

Differences between primates and rodents during normal neurogenesis are key to our current model predictions of increased sensitivity to ethanol's effects in primates when compared to rodents. We have previously identified key interspecies differences in the process of normal neocortical development through computational models (Gohlke et al., 2007). For example, in primate species neurogenesis lasts 10–14 times longer than in rodents (6 and 7 days in the mouse and rat compared to 60 and 84 days in the rhesus monkey and

human) (Rakic and Kornack, 2001). In addition, the cell cycle length is approximately twice as long in the rhesus monkey compared with the mouse or rat (Kornack and Rakic, 1998; Nowakowski et al., 1989; Takahashi et al., 1995), and the monkey and human neocortices are estimated to contain over 400 and 1,000 times as many neurons as the rat neocortex, respectively (Duffell et al., 2000; Lidow and Song, 2001; Pakkenberg and Gundersen, 1997).

To predict cross-species differences, we assume constant doses across species and neurogenesis; therefore, the critical dose metric (peak daily dose) and critical biological process (neurogenesis) is consistent across species. For our current model predictions, the increased number of cell cycles in primate species in the beginning stages of neurogenesis is particularly important, when neuronal progenitor population growth is virtually exponential, as the percentage of cells leaving the proliferative population is minimal (Kornack and Rakic, 1998). This creates a steeper slope of neuronal production during mid to late neurogenesis in primates when compared to rodents (Gohlke et al. 2007). Significant ethanol-induced lengthening of the cell cycle occurs specifically during the early stages of neurogenesis (Miller and Kuhn, 1995). Therefore, our computational models predict the rhesus monkey and human may be more sensitive to ethanol-induced neuronal loss, as the early stage of neurogenesis is the critical period responsible for the massive increase in neocortical neuronal progenitor number, necessary for the greatly enlarged neocortex seen in the primate species. In fact, primates have devoted a much greater portion of their resources to the development of a highly specialized neocortex, the center of our unique intelligence. This evolutionary concept is explored in more detail in a previous publication (Gohlke et al., 2007).

Applying Our Model Predictions to Risk Assessment

To examine the relevance of our model results in the broader context needed for utilization as a risk assessment tool, we compiled and critically compared in vivo studies in the rat, monkey, and human investigating neurodevelopmental effects of low to moderate doses of prenatal ethanol exposure (Fig. 4 and Table 1). Lowest Observed Adverse Effect Levels (LOAELs) are compared across species. Epidemiological measures of average alcohol exposure are compared to peak BECs by assuming an average of 1 drink/day would result in a peak BEC of 20 mg/dl (Driscoll et al., 1990). This analysis shows rat, human, and non-human primate studies describe comparable effects at similar peak blood alcohol levels, and that significant effects are evident down to the lowest dose examined in humans and non-human primates of approximately 1 drink/day or 20 mg/dl daily peak BEC (Fig. 4). This analysis supports our model predictions using a Weibull dose-response function, which predicts a significant effect in the human at a peak daily BEC between 10 and 20 mg/dl.

In addition, this analysis of the current literature highlights the paucity of dose-response data available for risk assessment characterization in any species, as well as the limitations of epidemiological research in deriving an accurate measure of dose, particularly at lower levels of exposure as it may be uncommon for a person to drink less than one standard drink at a time, and questionnaires used in epidemiological analyses do not accurately reflect this type of exposure even if it does occur (Kerr et al., 2005; Stockwell et al., 2004). Therefore, our model predictions suggesting primates are more sensitive to ethanol-induced neurodevelopmental toxicity cannot be corroborated or refuted based on this comparative analysis of current literature. Alternatively, it highlights the need for data using a lower dosing paradigm in primate species or better ascertainment of dose in epidemiological analyses. In addition, this analysis highlights the need to experimentally explore other potential sources of species-specific parameters in both kinetic and dynamic processes. In vitro systems may offer some insight into cross-species considerations.

In Vivo:In Vitro Comparisons

The Lowest Observed Adverse Effect Levels (LOAELs) for in vivo studies of rat, monkey, and human are from studies presented in Figure 4 and Table 1. The LOAEL for human studies is 1 drink per day or a peak BEC of 20 mg/dl/day. However, it should be noted no NOAEL has been determined and in fact subtle neurobehavioural deficits have been described down to the lowest non-zero dose level in two large prospective cohort studies undertaken in Seattle and Pittsburgh using non-linear regressions (Goldschmidt et al., 1996; Sampson et al., 2000a). Evaluation of in vitro analyses were confined to cultures of neural or glial progenitor cells as this would be the most relevant for developmental neurotoxicity estimations. Rodent-derived in vitro studies in neural stem or progenitor cells do not show significant effects on viability or apoptosis at medium concentrations below 120 mg/dl (25 mM), yet premature differentiation may occur at this concentration (Ma et al., 2003; Prock and Miranda, 2007; Tateno et al., 2005). Studies done in primary cultures of rat astroglial cells suggest effects at a 23-mg/dl (5 mM) medium concentration (Guizzetti and Costa, 1996; Kotter et al., 2000). These studies show a dose-response relationship based on the percent inhibition of ^{3}[H]-thymidine incorporation after 24-h exposure to ethanol in rat primary astrocytes (Guizzetti and Costa, 1996; Kotter et al., 2000). Furthermore, a similar dose-response relationship was found in human fetal astrocytes (Guizzetti et al., 2003). LOAEL values from in vitro studies in the human are based on a study looking at effects of ethanol in cultured human undifferentiated neural stem cells (Hao et al., 2003). Human neural stem cells may be acutely sensitive to ethanol exposure in culture, as extremely low concentrations (0.1 mM or 0.46 mg/dl) decrease viability in these cells (Hao et al., 2003). This is an interesting finding considering ethanol is endogenously produced by human intestinal flora (as a metabolic by-product), and present in human blood at an average background level of 0.15 mg/dL (HSDB, 2002). Importantly, neural stem cell culture systems derived from rodents do not show this increased sensitivity to ethanol exposure (Camarillo et al., 2007; Prock and Miranda, 2007; Tateno et al., 2005). However, it is unclear how differences in the culture methods may affect this sensitivity to ethanol.

Quantitation of variability in toxicodynamic model predictions is an extremely important endeavor in order

to fully understand risk estimates to populations (Hattis, 1996; Kimmel et al., 1995). Here we have developed methods for comparing contributions of variability in mouse, rat, and primate neocortical neurogenesis using a biologically based computational model. This endeavor also serves as a robust methodology for building mechanistic hypotheses of neurodevelopmental toxicity.

Ethanol-induced developmental neocortical neurotoxicity is characterized by a range of cellular effects depending on the dose and time of exposure. However, the changes in cell number that our model construct tracks, may be a sufficient explanation to describe the key sensitive toxic effects of ethanol for risk assessment purposes. This mode of action hypothesis has been used to quantitatively evaluate methylmercury neurodevelopmental toxicity and assess interspecies differences in a biologically based model framework (Lewandowski, 2000; Lewandowski et al., 2003b). Application of other neurodevelopmental toxicants such as radiation, organophosphate pesticides, and benomyl is being explored (DeFrank et al., 2004; Faustman et al., 2005). This mode of action modeling methodology has the potential to vastly improve the usage of scientific data in the developmental toxicology risk assessment arena by providing a quantitative framework in which cellular effects can be linked to an adverse neurodevelopmental outcome.

In conclusion, for interspecies extrapolation of neurotoxicity data for risk assessment purposes, it is particularly pertinent to acknowledge the vast interspecies differences in the neocortex. Important behavioral attributes, such as executive functioning or decision-making skills, associated with the enlarged human neocortex are absent in the primary animal models, such as the rat and rabbit, used in developmental toxicology studies. Our toxicodynamic model allows interspecies comparisons of toxicant-induced effects on the key processes of proliferation, differentiation, and death during the development of this important brain region.

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