Neurotoxicology and Teratology 35 (2013) 7–13

Contents lists available at SciVerse ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Different patterns of regional Purkinje cell loss in the cerebellar vermis as a function of the timing of prenatal ethanol exposure in an ovine model ☆

Onkar B. Sawant [a], Emilie R. Lunde [a], Shannon E. Washburn [a,*], Wei-Jung A. Chen [b], Charles R. Goodlett [c], Timothy A. Cudd [a]

[a] Department of Veterinary Physiology and Pharmacology and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, 77843, USA
[b] Department of Neuroscience and Experimental Therapeutics, Texas A&M Health Science Center, College Station, Texas, 77843, USA
[c] Department of Psychology, Indiana University-Purdue University Indianapolis, Indiana, 46202, USA

ARTICLE INFO

Article history:
Received 3 May 2012
Received in revised form 10 October 2012
Accepted 13 November 2012
Available online 27 November 2012

Keywords:
Cerebellum
Ovine
Ethanol
Teratogenicity
Purkinje cells

ABSTRACT

Studies in rat models of fetal alcohol spectrum disorders have indicated that the cerebellum is particularly vulnerable to ethanol-induced Purkinje cell loss during the third trimester-equivalent, with striking regional differences in vulnerability in which early-maturing regions in the vermis show significantly more loss than the late-maturing regions. The current study tested the hypothesis that the sheep model will show similar regional differences in fetal cerebellar Purkinje cell loss when prenatal binge ethanol exposure is restricted to the prenatal period of brain development equivalent to the third trimester and also compared the pattern of loss to that produced by exposure during the first trimester-equivalent. Pregnant Suffolk sheep were assigned to four groups: first trimester-equivalent saline control group, first trimester-equivalent ethanol group (1.75 g/kg/day), third trimester-equivalent saline control group, and third trimester-equivalent ethanol group (1.75 g/kg/day). Ethanol was administered as an intravenous infusion on 3 consecutive days followed by a 4-day ethanol-free interval, to mimic a weekend binge drinking pattern. Animals from all four groups were sacrificed and fetal brains were harvested on gestation day 133. Fetal cerebellar Purkinje cell counts were performed in an early-maturing region (lobules I–X) and a late-maturing region (lobules VIc–VII) from mid-sagittal sections of the cerebellar vermis. As predicted, the third trimester-equivalent ethanol exposure caused a significant reduction in the fetal cerebellar Purkinje cell volume density and Purkinje cell number in the early-maturing region, but not in the late-maturing region. In contrast, the first trimester-equivalent ethanol exposure resulted in significant reductions in both the early and late-maturing regions. These data confirmed that the previous findings in rat models that third trimester-equivalent prenatal ethanol exposure resulted in regionally-specific Purkinje cell loss in the early-maturing region of the vermis, and further demonstrated that first trimester ethanol exposure caused more generalized fetal cerebellar Purkinje cell loss, independent of the cerebellar vermal region. These findings support the idea that prenatal ethanol exposure in the first trimester interferes with the genesis of Purkinje cells in an unselective manner, whereas exposure during the third trimester selectively kills post-mitotic Purkinje cells in specific vermal regions during a vulnerable period of differentiation and synaptogenesis.

Published by Elsevier Inc.

1. Introduction

Fetal alcohol spectrum disorders (FASD) is an umbrella term encompassing the full range of effects that can occur in an individual whose mother consumed ethanol during pregnancy. These include effects on physical, behavioral or cognitive development that can persist as lifelong disabilities. The most severe end of the spectrum is fetal alcohol syndrome (FAS) (Abel, 1984; Streissguth et al., 1980; Warren and Bast, 1988; Warren et al., 2001), for which facial abnormalities, growth deficits and central nervous system (CNS) abnormalities are the defining diagnostic features. Despite efforts to educate the public about FASD, the prevalence of ethanol consumption in women of child-bearing age has remained essentially the same (Anon., 2003; Caetano et al., 2006; CDC, 2004; NIAAA, 2000). Ethanol is a neurotoxic teratogen and has the potential to cause damage in many regions of the brain (Behnke and Eyler, 1993; Caetano et al., 2006; Gladstone et al., 1996; Riley et al., 2004; Streissguth et al., 1980). Postmortem reports and neuroimaging studies in children clinically affected by the heavy prenatal ethanol

☆ Supported by NIAAA Grant AA017120 and AA10940 (T.A.C.) and K08AA18166 (S.E.W.). All or part of this work was done in conjunction with the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), which is funded by grants from the National Institute on Alcohol and Alcohol Abuse (NIAAA). Additional information about CIFASD can be found at www.cifasd.org.

* Corresponding author at: Department of Veterinary Physiology and Pharmacology and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4466, USA. Tel.: +1 979 845 1993; fax: +1 979 845 6544.
E-mail address: swashburn@cvm.tamu.edu (S.E. Washburn).

0892-0362/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ntt.2012.11.001

exposure have shown smaller head and brain size (Archibald et al., 2001; Clarren et al., 1978; Jones and Smith, 1973; Mattson et al., 1996; Peiffer et al., 1979; Swayze et al., 1997). Human studies have reported structural abnormalities and physiological dysfunction in several brain regions, including the cerebral cortex, basal ganglia, thalamus and hypothalamus, hippocampus, and corpus callosum (Archibald et al., 2001; Mattson et al., 1996; Riley et al., 1995; Sowell et al., 2002, 2008; Swayze et al., 1997), but based on both human and animal studies the cerebellum appears to be particularly vulnerable to ethanol exposure during development (Autti-Ramo et al., 2002; Bauer-Moffett and Altman, 1975, 1977; Bonthius and West, 1990; Goodlett et al., 1990a, 1990b; Hamre and West, 1993; Light et al., 2002b; Ramadoss et al., 2007a, 2007b; Washburn et al., 2013; West et al., 1990).

The timing of ethanol exposure during pregnancy is an important determinant of the type and extent of fetal brain damage that occurs (Clarren et al., 1978; Coles et al., 1991; Renwick and Asker, 1983; Riley et al., 2004). Human and animal studies have indicated that the developing cerebellum is vulnerable throughout gestation. Experimental studies in neonatal rats have shown that daily binge-like patterns of ethanol exposure, during the early postnatal period of brain development that is comparable to that of the human third trimester, produced dose-dependent loss of post-mitotic cerebellar Purkinje cells when peak blood ethanol concentrations (BECs) exceeded 150 mg/dl (Cragg and Phillips, 1985; Goodlett and Eilers, 1997; Goodlett et al., 1998; Hamre and West, 1993; Light et al., 2002a, 2002b; Maier and West, 2001a, 2001b; Marcussen et al., 1994; Pauli et al., 1995; Pierce et al., 1989). In contrast, when daily binge-like ethanol exposure was given throughout gestation in rats (when brain development is equivalent to that of the human first and second trimesters) in doses of 2.25, 4.5, or 6.5 g/kg/day (producing mean peak BECs of 136, 290, and 422 mg/dl, respectively), only the high dose (6.5 g/kg) resulted in a Purkinje cell loss (Maier and West, 2001b). In addition, direct comparisons of binge-like ethanol exposure in rats during the second trimester-equivalent versus the third trimester-equivalent, in which efforts were made to match the resulting BECs across the two periods, showed significantly greater reductions with the third trimester-equivalent exposure (Marcussen et al., 1994). In contrast, studies in a sheep model and a nonhuman primate model of binge-like prenatal ethanol exposure reported significant reductions in cerebellar Purkinje cell numbers regardless of whether exposure was during the period of brain development equivalent to that of the human first trimester, third trimester, or all three trimester-equivalents (Bonthius et al., 1996; Ramadoss et al., 2007a, 2007b; Washburn et al., 2013).

The findings in the sheep model, in which brain development over all three trimesters equivalent occurs *in utero*, raise interesting questions as to why comparable Purkinje cell loss was observed with different exposure periods or durations across pregnancy. The stages of cerebellar development and the mechanisms of Purkinje cell loss resulting from ethanol exposure are markedly different between the first- and third trimester-equivalent. In terms of Purkinje cell development, the first trimester exposure occurs prior to Purkinje cell genesis and so will affect the precursor cells, whereas the third trimester exposure occurs after the genesis of Purkinje cells, during their post-mitotic differentiation. Hence, the specific nature of temporal windows of vulnerability to ethanol-induced cerebellar cell loss across different stages of brain development has not been resolved, including whether the temporal and regional profiles of cerebellar vulnerability to ethanol may vary across species.

The possibility that the regional differences in the fetal cerebellar Purkinje cell loss in response to ethanol exposure may be related to the chronology of lobular development was first proposed by Bauer-Moffett and Altman (1975, 1977). Experimentally, Pierce and colleagues (1989) showed that postnatal ethanol exposure from day 4 to 10 in Sprague–Dawley rats resulted in more severe Purkinje cell loss in lobule I than in lobules V, VII, VIII, and IX of the cerebellar vermis. Bonthius and West (1990) then demonstrated that the Purkinje cells that were more mature at the time of ethanol exposure were more vulnerable than the less mature Purkinje cells, which could account for a substantial regional variation in the vermis in the extent of alcohol-induced Purkinje cell loss in the third trimester-equivalent rat model. A single episode of binge-like ethanol exposure on postnatal day 4 caused Purkinje cell loss in lobule I to V, IX and X but not in lobules VI–VII, and this differential vulnerability was correlated with differences in the timing of Purkinje cell dendritic outgrowth, which is initiated in lobules VI–VII significantly later than in lobules I–IV and IX–X (Goodlett et al., 1990a, 1990b). The relatively greater vulnerability of the "early-maturing" lobules of the cerebellum in the neonatal rat model is consistent with the findings by Hamre and West (1993) who showed that when binge-like exposure was limited to two days starting on different days between postnatal days 4–9, the greatest Purkinje cell loss was observed when ethanol exposure occurred on postnatal days 4–5 and no significant cell loss was observed when the exposure began after postnatal day 7 (Hamre and West, 1993).

The goal of the present study was to use the sheep model to better resolve the extent to which vulnerability to prenatal ethanol-induced Purkinje cell loss depends both on the developmental timing of exposure and on region within the vermis. A binge ethanol exposure paradigm was restricted to either the entire first trimester-equivalent or the entire third trimester-equivalent, and mid-sagittal sections of the cerebellar vermis were divided into the early-maturing region, defined as lobules I and X, and the late-maturing region, defined as portions of lobules VIc and VII (see Fig. 1). We hypothesized that both the early- and late-maturing cerebellar regions would be vulnerable to prenatal ethanol exposure but that the third trimester exposure would produce the Purkinje cell loss in the early-maturing but not in the late-maturing lobules, whereas the first trimester exposure would produce a generalized Purkinje cell loss irrespective of the vermal region.

2. Methods

2.1. Animals and breeding

The experimental procedures were approved by the Institutional Animal Care and Animal Use Committee at Texas A & M University. Suffolk ewes (aged 2 to 6 years) were bred under controlled conditions as described elsewhere (Ramadoss et al., 2006). The day of mating (the day that the ewes were marked by the ram) was designated as gestational day (GD) 0. Ewes were then maintained in an environmentally regulated facility (22 °C and a 12:12 light/dark cycle) where they remained for the duration of the experiments. Animals were fed 2 kg/day of a "complete" ration (Sheep and Goat Pellet; Producers Cooperative, Bryan TX) and they consumed all of the feed offered.

2.2. Treatment groups

There were 4 treatment groups (total $n=29$) in this study: first trimester-equivalent saline control group (T1Sal) ($n=7$), first trimester-equivalent ethanol group (1.75 g/kg/day) (T1Eth) ($n=7$), third trimester-equivalent saline control group (T3Sal) ($n=7$), and third trimester-equivalent ethanol group (1.75 g/kg/day) (T3Eth) ($n=8$). This study modeled intermittent binge drinking over the first trimester-equivalent and the third-trimester-equivalent, respectively,

2.3. First trimester-equivalent groups

On GD 4, the beginning of the first trimester-equivalent in this species, an intravenous catheter (16 ga., 5.25 inch Angiocath™; Becton Dickinson, Sandy, UT) was placed into the jugular vein of the ewes. Beginning on GD 4, ethanol (1.75 g/kg) or saline was administered intravenously over a 1-hour period via a peristaltic pump (Masterflex, Model 7014–20; Cole-Parmer, Niles, IL). The ethanol solution was prepared by adding 95% ethanol to sterile 0.9% saline to achieve a 40% w/v ethanol