Developmental neurotoxicity: do similar phenotypes indicate a common mode of action? A comparison of fetal alcohol syndrome, toluene embryopathy and maternal phenylketonuria

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Abstract

Developmental neurotoxicity can be ascribed to in utero exposure to exogenous substances or to exposure of the fetus to endogenous compounds that accumulate because of genetic mutations. One of the best recognized human neuroteratogens is ethanol. The Fetal Alcohol Syndrome (FAS) is characterized by growth deficiency, particular facial features, and central nervous system (CNS) dysfunctions (mental retardation, microencephaly and brain malformations). Abuse of toluene by pregnant women can lead to an embryopathy (fetal solvent syndrome, (FSS)) whose characteristics are similar to FAS. Phenylketonuria (PKU) is a genetic defect in phenylalanine (Phe) metabolism. Offspring of phenylketonuric mothers not under strict dietary control are born with maternal PKU (mPKU), a syndrome with similar characteristics as FAS and FSS. While ethanol has been shown to cause neuronal death, no such evidence is available for toluene or Phe and/or its metabolites. On the other hand, alterations in astrocyte proliferation and maturation have been found, mostly in in vitro studies, which may represent a potential common mode of action for at least some of the CNS effects found in FAS, mPKU, and FSS. Further in vivo and in vitro studies should validate this hypothesis and elucidate possible molecular targets. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Fetal alcohol syndrome; Toluene embryopathy; Maternal phenylketonuria; Glial cells; Neuronal death

1. Introduction

Developmental neurotoxicity can be ascribed to in utero exposure to exogenous substances or to exposure of the fetus to endogenous compounds that accumulate because of genetic mutations. One of the best recognized human neuroteratogens is ethanol. Offspring of alcoholics often present a syndrome (fetal alcohol syndrome or FAS) whose principal features include growth deficiency, particular facial features, and, of most concern because irreversible, central nervous sys-
tem (CNS) dysfunction (mental retardation, microencephaly and brain malformations). Abuse of the common solvent toluene (‘toluene sniffing’) by pregnant women can lead to an embryopathy also referred to as fetal solvent syndrome (FSS). Characteristics of toluene embryopathy include craniofacial features similar to FAS, growth retardation and CNS dysfunctions, such as microencephaly, brain malformations and mental retardation. Phenylketonuria (PKU) is a genetic defect in phenylalanine (Phe) hydroxylase, an enzyme that converts Phe to tyrosine, leading to an accumulation of Phe and its metabolites. Offspring of phenylketonuric mothers not under strict dietary control are born with maternal PKU (mPKU), a syndrome characterized by dysmorphic facies, growth retardation and CNS abnormalities, such as microencephaly and mental retardation. Thus, exposure to two unrelated compounds, alcohol and toluene, and a genetic defect, can all lead to developmental effects which display very similar phenotypes.

An understanding of the mode of action underlying such developmental effects would be of great interest, as it may shed light, among others, on possible therapeutic interventions. The term ‘mode of action’ was coined to describe a series of events supported by a body of scientific knowledge, that provides a biologically plausible explanation of causality for a given toxic effect within a context of dose and duration of exposure, and susceptibility of target tissue (Bogdanffy et al., 2001). It is contrasted to the term ‘mechanism of action,’ in which all of the key events from the molecular to the organismal level have been identified. Identification of similar modes of action for toxicants may also vastly improve the risk assessment process (Hoeft, 2001). In particular, while in vivo and in vitro studies have shown that ethanol can cause apoptotic neuronal cell death, the evidence is more limited for mPKU and for toluene. Inhibition of the proliferation of astroglial cells is emerging as one important mechanism by which alcohol affects brain development, and in vitro evidence suggests that Phe and some of its metabolites, as well as toluene, may exert a similar effect in vitro. This paper briefly reviews some of the key features of FAS, mPKU and toluene embryopathy, as well available evidence to address the hypothesis that alterations in astrocyte development may represent a common mode of action to explain at least some of the CNS effects found in these similar syndromes.

2. Fetal alcohol syndrome

Maternal intake of ethanol during pregnancy is detrimental to human fetal development (Jones and Smith, 1973; Streissguth et al., 1980). Offspring of alcoholics often present a syndrome (FAS) whose principal features include CNS dysfunctions (mental retardation, microencephaly, brain malformations), growth deficiency, and particular facial features (Streissguth et al., 1980). The CNS deficits of FAS appear to be long-lasting, since various studies have shown that they persist in young adults born with FAS, even if other symptoms (growth retardation, facial characteristics) have subsided (Streissguth et al., 1991; Lemoine and Lemoine, 1992; Spohr et al., 1993). Cognitive and intellectual deficits in children born to alcoholic mothers have been described even in the absence of the typical characteristics of FAS (Streissguth et al., 1980). FAS is now considered a leading cause of mental retardation in the general population (Abel and Sokol, 1986), and its inci-
idence in the US has been reported to range between 0.67/1000 and 9.1/1000 (Abel, 1988; CDC, 1995; Sampson et al., 1997).

A very large number of studies have been conducted in laboratory animals to gain an understanding of the characteristics and mechanisms of ethanol’s developmental neurotoxicity, and their results have been summarized in several books and reviews (West, 1986; Watson, 1992; Abel, 1988). In vivo studies in rodents have utilized both prenatal and postnatal exposures, to mimic human exposure during the first and early second trimester, and the late second and third trimester of early postnatal life, respectively. Several investigations have shown that ethanol can cause selective losses of hippocampal pyramidal cells or cerebellar Purkinje or granule cells (Miller, 1995; Maier et al., 1999). Administration of ethanol to rats on postnatal day seven has been shown to cause severe apoptotic neuronal death in the hippocampus and cerebral cortex (Ikonomidou et al., 2000). In vitro studies have indicated that ethanol can cause apoptotic cell death in neurons either directly (Oberdoerster and Rabin, 1999), or indirectly, by inhibiting the protective action of trophic factor such as glutamate through NMDA receptors (Bhave and Hoffman, 1997), IGF-1 (Cui et al., 1997) or acetylcholine (Castoldi et al., 1998).

Several investigations have also shown that ethanol, both in vitro and in vivo, can significantly affect glial cells (Phillips, 1992; Snyder, 1996; Guerri and Renau-Piqueras, 1997; Guerri et al., 2001). Abnormalities in glial development have been shown in human with FAS, as well as in primates and rats exposed to ethanol during development (Clarren, 1986; Miller and Potempa, 1990). The finding that microencephaly is strongly associated with ethanol exposure during the brain growth spurt (Samson, 1986), a period characterized by rapid glial cell proliferation and maturation, suggests a potential effect of ethanol on the proliferation, growth and maturation of glia. Several studies have investigated the effects of ethanol on astrocytes or other glial cell lines in culture. In addition to various effects on cytoskeletal proteins and other enzymes (Guerri et al., 2001), ethanol has been found to exert a profound inhibitory effect on glial cell proliferation (reviewed in Guizzetti et al., 1997). In particular, proliferation induced by IGF-1 and cholinergic muscarinic agonists is inhibited by ethanol at low relevant concentrations (10–100 mM; Resnicoff et al., 1994; Guizzetti and Costa, 1996).

3. Maternal phenylketonuria

Phenylketonuria is a genetic defect in Phe hydroxylase, an enzyme that converts Phe to tyrosine. In humans, PKU is characterized by a plasma Phe level above 1.2 mM (Rezvani, 1996), however, plasma Phe levels as high as 6 mM have been reported (Swaiman and Wu, 1984). At birth, an individual with classic PKU (cPKU) is clinically normal and, as a result of early diagnosis and treatment with a diet low in Phe, 97% of all diagnosed phenylketonuric children are intellectually normal (Williamson et al., 1981). If left untreated, however, cPKU results in mental retardation and seizures (Menkes, 1995; Rezvani, 1996). Discontinuation of dietary therapy after adolescence has essentially no adverse effect on the nervous system and, as a result, strict dietary control is often not maintained after 17 years of age (Potoczniak and Widhalm, 1994; Wilkinson and Holbrook, 1998). Thus, although in the past individuals with cPKU did not reproduce due to their severe mental retardation, phenylketonuric women, having normal fertility, are having high-risk pregnancies; offspring of phenylketonuric mothers not under dietary control are born with maternal PKU (mPKU) (Hanley et al., 1987; Levy and Ghavami, 1996; Rouse et al., 1997). These individuals, although genotypically normal, have severe CNS dysfunctions, including mental retardation, microcephaly and seizure. For example, 73–100% of offspring from mothers with untreated cPKU were reported to have microcephaly, and 92–94% were classified as being mentally retarded (Lenke and Levy, 1980; Lipson et al., 1984). In addition to CNS effects, individuals diagnosed with mPKU also present facial dysmorphisms, congenital heart abnormalities, and growth retardation (Levy and Ghavami, 1996;
Rouse et al., 1997, 2000). Though a strict dietary regimen with a Phe-free diet would prevent mPKU, this is often difficult to achieve (Sheard, 2000), and mPKU is a continuing cause of concern (Mowat et al., 1999; Koch et al., 2000).

Phe is primarily metabolized to tyrosine; however, in hyperphenylalanimimetic individuals, the conversion of Phe to nontyrosine derivatives becomes significant. Thus, Phe is decarboxylated to phenylethylalanine (PEA), 90% of which is oxidized to phenylacetic acid (PAA), and the remainder to mandelic acid (MA). In addition, Phe can also be transaminated to phenylpyruvic acid (PPA) which is converted to phenylactic acid (PLA) and hydroxyphenylacetic acid (HPA). These Phe metabolites have all been shown to be significantly elevated in hyperphenylalaninemic individuals, with urinary excretion increased from 6- to 16-fold (Tuchman et al., 1985; Kaufmann, 1989; Clemens et al., 1990). Whether Phe itself, or one of its metabolites, is responsible for the CNS dysfunctions associated with mPKU is unknown. While limited evidence in humans suggests that Phe, most likely in combination with one or more of its metabolites, may be the agent responsible for the CNS abnormalities (Levy and Ghavami, 1996), animal studies suggest that PAA, whose levels in brain can reach 0.4–3 mM, may be the toxic metabolite (Wen et al., 1980; Loo et al., 1980; Manabe and Ohsawa, 1993).

The mechanism(s) of the CNS dysfunctions associated with mPKU is unknown. It is evident, however, that exposure to high levels of Phe, or one of its metabolites, is toxic to the developing brain, whereas the fully developed brain is essentially unaffected (Potocnik and Widhalm, 1994). Thus, there appears to be a developmentally restricted window of vulnerability to hyperphenylalaninemia. Exposure to high levels of Phe during the brain growth spurt results in mental retardation and decreased brain size (Lipson et al., 1984; Levy and Ghavami, 1996; Roricht et al., 1999). In addition, studies in children with mPKU have revealed a loss of neurons (Lacey and Terplan, 1987; Levy et al., 1996), and animal studies have suggested a hyperphenylalaninemia-mediated increase in cell death in the developing brain (Reynolds et al., 1993).

The hypothesis that the microencephaly and neuronal loss associated with mPKU may be due, at least in part, to enhanced neuronal cell death and decreased astrocyte proliferation, was recently tested (Oberdoerster et al., 2000). Though autopsies and magnetic resonance imaging of children with mPKU have revealed a loss of neurons (Lacey and Terplan, 1987; Levy et al., 1996), a direct toxic effect of Phe or its metabolites on neurons appears unlikely. Silberberg (1967) reported that a 10-day exposure to 9 mM Phe or 1.1 mM PAA was not toxic to mixed rat cerebellar neurons. Furthermore, neither Phe nor any of its metabolites (PPA, HPA, MA, PEA and PLA at 0.5 mM; PAA at 5 mM) had any cytotoxic effect on rat cerebellar granule cells or SH-SY5Y human neuroblastoma cells (Oberdoerster et al., 2000).

On the other hand, Phe was found to induce vacuole formation in glial cell populations following prolonged exposure (Silberberg, 1967), suggesting that Phe and/or its metabolites may have an adverse effect on glial cells during development. Oberdoerster et al. (2000) recently tested the effect of Phe and its metabolites on the proliferation of astroglial cells in vitro. A reduction in the number of glial cells during development would indeed not only result in microencephaly, but also may contribute to a corresponding loss of neurons. In fact, proliferation of rat cortical astrocytes, human fetal astrocytes and a human astrocytoma cell line was found to be sensitive to the inhibitory effect of Phe, PEA and PAA (Oberdoerster et al., 2000).

4. Toluene embryopathy

Toluene (methylbenzene) is an aromatic hydrocarbon commonly used as a starting material in the manufacture of a variety of organic compounds, and as a solvent or thinner in numerous industrial products including paints, lacquers, enamel, varnish, glue, guns, fat and resins (Low et al., 1988). Production of toluene for 1994 in the United States alone has been estimated at more than three million tons (Greenberg, 1997).
Exposure in humans may occur primarily in occupational settings, where safe levels in air are set at 100 ppm. For environmental exposure of the general population, an inhalation reference concentration of 0.1 ppm was developed by the US EPA, primarily based on a study in occupationally exposed workers (Greenberg, 1997). The primary target for toluene toxicity is believed to be the CNS (Benignus, 1981). Behavioral abnormalities have been reported in humans following acute (Echeverria et al., 1989) or chronic (Foo et al., 1988) exposure to toluene. Such effects include alterations in mood, memory loss and a decreased hearing capacity. Animal studies have confirmed that repeated toluene exposure causes neuronal damage, reactive gliosis and alterations in cognitive behavior (Huang et al., 1992; Korbo et al., 1996).

In addition to occupational and environmental exposure, in the past few decades toluene has become popular as a recreational drug; ‘solvent-sniffing’ or other inhalation of toluene in commercial products has been widely reported in the literature and encompasses exposure to toluene levels that far exceed exposure limits and can reach concentrations as high as 500–5000 ppm (Ron, 1986). Toluene use can lead to severe CNS abnormalities including cerebellar degeneration, cortical atrophy, and impaired mental and intellectual performance (Streicher et al., 1981; Lazar et al., 1983; Damasceno and de Capitani, 1994).

A number of studies on the teratogenicity and developmental toxicity of toluene have been carried out in various animal species (Ono et al., 1995; Wilkins-Haug, 1997). Adverse effects found in these studies include ossification delay, skeletal malformations and, in particular, fetal growth retardation. Over the past twenty years evidence has been emerging, supported by several case-reports, that toluene may be a significant human teratogen, and that the CNS is a primary target for its developmental toxicity (Wilkins-Haug, 1997; Jones and Balster, 1998). The first report dates to 1979 (Toutant and Lippmann, 1979), when a newborn (born from a toluene abusing mother) was described, as displaying low birth weight, microcephaly, a flat nasal bridge, short palpebra fissures, and other abnormal features. Since then, several other reports have appeared in the literature describing offspring of toluene-abusing women and delineating the phenotypic characteristics of a toluene embryopathy. Growth retardation and microcephaly are the major effects seen in newborns, accompanied by a number of dysmorphic features such as deep set eyes, low set ears, flat nasal bridge, micrognathia and small fingernails (Hersch et al., 1985; Hersch, 1989; Arnold et al., 1994; Pearson et al., 1994). As the children mature, developmental delay, language impairment, hyperactivity, cerebellar dysfunction and postnatal growth retardation become evident (Hersch et al., 1985; Arnold et al., 1994).

The similarities of the effect of developmental exposure to toluene and those observed in the fetal alcohol syndrome (FAS) are striking and were noted from the beginning; indeed, Toutant and Lippmann (1979) reported their case as fetal solvents syndrome (FSS). While, in some reported cases, concomitant exposure to alcohol and/or to other drugs of abuse (e.g. cocaine) could not be ruled out, the majority of the cases reported appear to be linked solely to exposure to toluene during pregnancy.

In recent years, a potential animal model for toluene embryopathy has been developed (Gospe et al., 1994, 1996; Gospe and Zhou, 1998). In this rat model, dams are exposed to toluene by gavage from day 6 to day 19 of gestation. In one study, fetuses were delivered by cesarean section on embryonic day (ED) 19 (Gospe et al., 1996). Exposed fetuses showed generalized growth retardation with significant reduction in body and organ weights including brain. In a follow-up study (Gospe and Zhou, 1998), pups exposed prenatally in an identical manner were examined on postnatal days (PND) 10 and 21. On day 10, there was a significant reduction in whole body, heart and kidney, but not liver and brain, weights. No differences between toluene-exposed animals and controls were observed in animals examined on PND 21. Morphometric analysis of the whole brain revealed significant alterations on ED 19 (Gospe et al., 1996; Gospe and Zhou, 1998). A reversal of this effect, however, was observed by PND 10 and 21. Decreases in forebrain protein, cholesterol and DNA contents, with a reduction
in forebrain cell nuclei were also found on ED 19; a reduction of DNA content and cell nuclei were still present on day 10, but were not apparent on PND 21. Alterations in some of these parameters were also found in the hindbrain, with a full recovery on PND 21. The only persistent effect noted in 21 day-old pups was a decrease in the forebrain cholesterol/DNA ratio, possibly indicating an effect of toluene on myelin development (Gospe and Zhou, 1998).

In a follow-up study (Gospe and Zhou, 2000) pregnant rats were exposed to toluene during day 6–21 of gestation and pups were sacrificed at PND 21. Toluene exposure resulted in significant abnormalities of cortical cytoarchitecture and neuronal generation, with a 12.6% loss in the number of cortical neurons, though it is not known whether such change is due to a reduction in neuronal generation and/or enhanced neuronal apoptosis. Indeed, no information could be found with regard to the potential of toluene to induce apoptotic death of neurons, either in vivo upon developmental exposure, or in vitro.

There is also some indication that toluene may have direct toxic effects on glia (Hansson et al., 1988; Gospe and Zhou, 2000). In addition, postnatal exposure to toluene (PND 4-10) resulted in a decrease in brain GFAP content in rat pups (Burry and Costa, unpublished). In vitro, toluene has been shown to inhibit ATPases in astrocytes (Naskali et al., 1994), and to inhibit proliferation of human astrocytoma cells and rat cortical astrocytes, stimulated by serum or insulin-like growth factor I (Burry and Costa, unpublished). Though limited and preliminary, these observations suggest that glial cells may be a target for developmental neurotoxicity of toluene.

5. FAS, mPKU and FSS: a common mode of action?

The similarities of FAS, mPKU and FSS are striking (Table 1). In fact, the three syndromes may be difficult to distinguish on clinical examination. Facial dysmorphology and growth retardation are quite similar and, in all cases, tend to improve with time. CNS effects, such as microcephaly and microencephaly, and mental retardation, are believed to be irreversible. The similarities have led to the suggestion that FAS, mPKU and FSS may share a common pathogenesis (Levy and Ghavami, 1996; Pearson et al., 1994). Yet, what these common mechanisms could be remains elusive. Alcohol, and its metabolites, as well as toluene, freely cross the placenta, suggesting a direct toxic effect on the developing fetus. While ethanol and toluene may share the common feature of interfering with the cell membrane, this may not be the case with Phe and/or its metabolites. Moreover, in the case of mPKU, the relative contribution of Phe or its metabolites to developmental toxicity is unclear. Rather than common mechanisms of teratogenesis, these agents may have different mechanisms of developmental pathogenesis with a common phenotypic endpoint. Alternatively, these agents may share similar ‘modes of action’, by targeting similar cell types or cellular processes, albeit with different biochemical or molecular mechanisms.

In the preceding sections we have pointed out at the ability of ethanol, toluene and Phe and its metabolites to affect neurons and glial cells, particularly astrocytes. With regard to neurons, there is ample evidence that ethanol causes loss of neurons upon in vivo exposure, as well as apoptotic neuronal death in vitro. In mPKU such evidence is weaker, and limited in vitro studies do not support a direct toxic effect of Phe and/or its metabolites on neuronal cells. A loss of neurons

Table 1
Comparison of clinical features found in FAS, mPKU and FSS

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<th>Clinical Features</th>
<th>FAS</th>
<th>mPKU</th>
<th>FSS</th>
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<td>Growth and development</td>
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<tr>
<td>Prenatal growth deficiency</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>Postnatal growth deficiency</td>
<td>++</td>
<td>+</td>
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<tr>
<td>CNS effects</td>
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<tr>
<td>Microcephaly</td>
<td>+</td>
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<tr>
<td>Mental retardation</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hyperactivity</td>
<td>+</td>
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<tr>
<td>Cranofacial anomalies</td>
<td>+</td>
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<tr>
<td>Cardiac disease</td>
<td>+</td>
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has been reported also upon developmental exposure to toluene, however, no information on the direct toxicity of toluene on neuronal cells is available.

Substantial in vivo and in vitro evidence indicates that ethanol can also affect developing astrocytes. In particular, ethanol can inhibit the proliferation of glial cells, an effect that would lead to a decreased number of astrocytes and contribute to the observed microencephaly. Though no information is available on astroglial cell loss upon developmental toluene exposure or in mPKU, limited in vitro studies suggest that toluene as well as Phe and its metabolites PEA and PAA may inhibit proliferation of astrocytes. Such effect may on one hand contribute to the ensuing microencephaly. Furthermore, an effect on glial cells may in turn affect the development of neurons, given the essential role of astrocytes in fostering the development and survival of neurons (Rudge, 1993; Haydon, 2001). Though the hypothesis of a central role for glial cells in the developmental neurotoxicity of FAS, mPKU and FSS remains speculative at this time, due mostly to the limited information available for the two latter syndromes, it may offer a working hypothesis to design further studies on possible common modes of action.

Acknowledgements

Research by the authors was supported by grants from the National Institutes of Health (ES-07033, ES-10120, AA-08154), and the European Union (QLK4-1999-01562).

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