Valproic acid-induced congenital malformations: Clinical and experimental observations

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ABSTRACT With a large number of epileptic women being in the childbearing age group, complications of pregnancy in epileptic patients are of concern. Epileptic women are treated with antiepileptic drugs (AED) whether they are pregnant or not. Contrary to prevailing opinion, recent data suggest that epilepsy per se contributes significantly to birth defects possibly because of the same genetic susceptibility that predisposes to epilepsy. Many of these defects closely resemble those attributed to exposure to AED. The syndromes attributed to various AED also considerably overlap with each other. Valproic acid (VPA) induces several minor and major malformations. The relative risk for spina bifida in VPA exposed pregnancies is nearly 20 times higher than that for the general population and about 10 times higher than that attributed to other anticonvulsants. Fetuses of experimental animals treated with VPA during pregnancy exhibit exencephaly unlike the human offspring in whom VPA induces spina bifida. The cranial and spinal malformations observed in humans and laboratory animals indicate that VPA has a preferentially deleterious effect on the neural crest. Several AEDs including VPA tend to lower maternal plasma folate levels. In view of the beneficial effects of periconceptional folate supplementation in prevention of neural tube defects (NTD), future research should be directed at the role of folate in the possible alleviation of VPA-induced NTD. It is also necessary to continue prospective studies to monitor the old and new AED prescribed and to evaluate the role of interactions between drugs used in combinations.

Key words: Valproic acid; congenital malformations; neural tube defects; craniofacial anomalies; humans, laboratory animals.

INTRODUCTION

The thalidomide episode of 1960s awakened the minds of scientific community to the immense possibility of apparently safe drugs used for different purposes being teratogenic. Valproic acid (VPA) or its sodium salt is one such agent which was claimed to be harmless at the time when it was first introduced in the market but later found to produce neural tube defects (NTD) and a spectrum of craniofacial anomalies termed fetal valproate syndrome (FVS). The antiepileptic property of this drug was first discovered in 1963 but soon became available for clinical use for primary and adjuvant control of several types of seizures. Many clinicians consider VPA the drug of choice for the treatment of generalized epilepsies including tonic-clonic and absence seizures (Brodie and Dichter, 1996; Aldredge, 2000). This review summarizes valproic acid-induced human malformations, major results of animal experiments and highlights the postulated mechanisms.

EPILEPSY AND PREGNANCY COMPLICATIONS

The prevalence of epilepsy is reported to be variable in different parts of the world (3-8 per 1000) but the trend indicates that the incidence is increasing (Yerbi, 1991; Nakane and Kaneko, 1992). Recent developments in diagnostic techniques, improvements in the quality of medical and surgical treatments and better social adjustment have made it possible for epileptic women to marry and bear children. About 40% of epileptic women are in the childbearing age group. Pregnancy in epileptic women is reported to be associated with increased frequency of seizures, and a decrease in plasma concentrations of antiepileptic drugs (AED). These women are also more prone to obstetric complications and premature labor than are...
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Table 1: Fetal abnormalities associated with maternal epilepsy*

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<th>Condition</th>
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<tr>
<td>Spontaneous abortion</td>
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<tr>
<td>Craniofacial abnormalities</td>
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<tr>
<td>Major birth defects (Neural tube defects, cleft lip, and cleft palate)</td>
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<tr>
<td>Minor anomalies</td>
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<td>Prematurity</td>
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<td>Hypoxia</td>
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<td>Pre- and postnatal growth deficiency</td>
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<td>Seizure</td>
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nonepileptic women (Yerbi et al., 1985, Yerbi, 1991). A Japanese study reported no changes during pregnancy in seizure frequency of 59% of epileptic women (Nakane and Kaneko, 1992). Whether or not epilepsy per se can induce a high incidence of congenital malformations (Table 1) has been debated (Ogawa et al., 1984; Hillemaa et al., 1985; Kallen et al., 1989) but inconclusively. At least one recent cohort study indicates that the prevalence of major malformations in infants of epileptic parents is twofold higher than that of the controls (Koch et al., 1992). The authors suggest that the same special genetic background of the fetus/parent that predisposes to epilepsy also renders the fetus uniquely susceptible to major and minor malformations and that the linkage between epilepsy and birth defects is stronger than that between AED and malformations. It is worth noting here that this study involved too few patients to make unequivocal conclusions.

ANTIEPILEPTIC DRUGS AND PREGNANCY OUTCOME

Janz and Fuchs (1964) were possibly the first to propose that AED therapy could be teratogenic but their study did not include controls, record review or clinical examination. Meadow (1968) reported six infants with cleft lip with or without cleft palate and unusual faces whose mothers had been exposed to anticonvulsants during pregnancy. The case control study of Speidel and Meadow (1972) was possibly the first which showed an increased incidence of birth defects (cleft lip/cleft palate and congenital heart diseases) in the offspring of epileptic women and according to them at least some of the increases were due to AED (particularly diphenylhydantoin) exposure. Kelly (1984) made an exhaustive review of the literature and concluded that the infants of women on AED therapy during pregnancy had 2 - 3 times higher risk for major malformations than the unexposed population.

Later a well planned and well executed multi-institutional collaborative study from Japan (Nakane et al., 1980) showed that the incidence of fetal malformations in AED exposed cases was five times greater than that in nonmedicated mothers. Cleft lip/cleft palate and cardiovascular malformations were found to be predominant and the incidence was even greater in AED-treated patients who had seizures during pregnancy. Another study, also by the same group (Kaneko et al., 1988) examined the reproductive toxic effects of as many as 13 AED in different types of seizure situations and observed no relationship between the malformations and the type of seizure. The rate of malformations in single drug treatment was much less (6.5%) than that for multiple AED (15.6%). Acetazolamide produced the least frequency (6.7%) and valproate caused the highest incidence (25.9%) as was the case with VPA plus carbamazepine (CBZ) and phenytoin (PHT) groups. No drug showed a dose-dependent increase in malformation rate. This and similar studies from other parts of the world (see Danksy and Finkel, 1991; Koch et al., 1992 for references) have now clearly established that almost every AED so far tested is teratogenic. Though all AED increase the number of minor anomalies, VPA, PHT and phenobarbital show specific malformations. It is now clear that the incidence of malformations in AED-treated epileptic cases is variable depending on the drug or combinations of drugs, etiology and type of epilepsy, seizure frequency during pregnancy and whether or not the mothers themselves had any malformations (Lammer et al., 1987; Kaneko et al., 1988). Among the several AED thus evaluated for their teratogenic potential, VPA stands out prominently because of the discovery that the association between VPA polypharmacy during pregnancy and congenital malformations in the offspring is stronger than that for any other combination of drugs (Meadow, 1991; Lindhout et al., 1992; Koch et al., 1992). Therefore it seems appropriate to conclude that VPA is strongly teratogenic and all other AED are mild teratogens.
VALPROIC ACID AND CONGENITAL MALFORMATIONS

Pharmacology and Placental Transport

Valproic acid (VPA) (di-n-propyl acetic acid) is a simple 8-carbon, 2-chain fatty acid of low molecular weight. It was first synthesized in 1882 by Burton (cited in Rimmer and Richens 1985). It was widely used as an organic solvent. Its antiepileptic effect was serendipitously discovered almost 100 years later by Meunier et al. (1963). The later investigators were testing the anticonvulsant property of a number of chemicals using valproic acid as a solvent and observed that every chemical thus tested exhibited the same anticonvulsant property and therefore they thought the effect was due to VPA.

The sodium and magnesium salts of VPA, the amide, dipropylacetamide as well as the acid itself are now marketed for human use. It was first used to treat epilepsy in France in 1964, in the UK in 1973 and in the USA in 1978. In Europe, it may be used for all types of seizures and in the USA for the treatment of absence seizures with or without other types of seizures. Although quite efficacious for the treatment of absence seizures, VPA has been demonstrated to be effective in a wide variety of seizure types often in combination with other AED. It is easily absorbed and widely distributed. A major portion is excreted as glucuronide in urine. β-Oxidation results in 2-en-VPA, and 3-keto-VPA both of which are major plasma metabolites in humans and laboratory animals (Nau and Loscher, 1984). Hydroxylation by microsomal cytochrome P-450 system results in the production of 3-OH, 4-OH and 5-OH metabolites of lesser concentration. Some small amounts of other unsaturated metabolites 3-en-VPA and 4-en-VPA are also observed. It has a half-life of 7-9 hr and easily excreted (Pinder et al., 1977). The half-life varies in laboratory animals between 0.5 hr and 2.0 hr. (Nau et al., 1991; Gordan et al., 1995). In humans, VPA has a longer half life (8 to 20 hrs) and appears to exhibit substantial inter-individual variations (Allredge, 2000). Differences in half life can only partly be explained by differences in protein binding since protein binding capacities are rather similar across species. These differences possibly relate to the differences in the genetic make up of the species and hence metabolic rates. This variation must also explain why sometimes high doses are required to produce pharmacologic or toxicologic effects comparable across species. The antiepileptic properties of VPA and its different metabolites seem to correlate well with their ability to elevate brain and nerve terminal concentrations of GABA.

Elevation in brain GABA concentration, selective increase of GABA levels in the synaptosomes, selective enhancement of the postsynaptic responses as well as direct effect on neuronal membranes related to potassium conductance etc. are suggested to be the mechanism of action of VPA but none has been proved conclusively (Rimmer and Richens, 1985; Biggs et al., 1992; Gordan et al., 1995). It interacts with a number of other anticonvulsants but because it does not induce hepatic enzymes, it is regarded as the most suitable AED for use in women on oral contraceptives. The parent drug seems to have higher anticonvulsant effect than any of its metabolites. Valproic acid crosses the placental barrier and attains concentrations in the cord blood higher than that of the maternal serum suggesting a significant fetal exposure during maternal therapy (Dickinson et al., 1979; Nau et al., 1984; Gordan et al., 1995). The two most remarkable side effects of VPA therapy are hepatotoxicity and teratogenicity.

Congenital Malformations

Human Studies

A number of case reports, case control studies and cohort (prospective and historical) studies reported in the literature describe congenital malformations in infants of epileptic mothers exposed to VPA and/or VPA plus other AED in utero. Dalens et al. (1980) were possibly the first to report on malformations of an infant whose mother was exposed to VPA during gestation. Several reports soon followed (Clay et al., 1981; Bailey et al., 1983; Bantzt, 1984; Tein and MacGregor, 1985; Thomas and Buchanan, 1981; Lammer et al., 1987). The birth defects attributed to VPA therapy included prominent forehead, flat nasal bridge, low set unusually shaped ears, hypertelorism, epicantthal folds, down slanting palpebral fissures, micrognathia, microcephaly, cardiovascular defects, duodenal atresia, renal hypoplasia, congenital hip dislocation, shortened forearm, digital malformations, nail hypoplasia, and vertebral malformations (Nakane et al., 1980; Kelley, 1984; Hansen et al., 1984; Tein and MacGregor, 1985; Ardinger et al., 1988). Three case reports of early 1980s also described isolated spina bifida in infants prenatally exposed to VPA (Gomez, 1981; Stanley and Chambers, 1982; Blaw and Woody, 1983). Preaxial reduction deformity of digits has also been reported to occur in fetal valproate syndrome (Sharony et al., 1993).

DiLiberti et al. (1984) described a characteristic pattern of minor facial anomalies in seven cases of prenatally VPA exposed infants and coined the term fetal valproate syndrome (FVS). This syndrome consists of epicantthal folds with an infraorbital crease, small upturned nose, flat nasal bridge, long upper lip with shallow philtrum, thin upper vermilion border and down-turned angles of mouth. Some of these infants also had other malformations such as hypospadias, strabismus and nystagmus. Ardinger et al. (1988) verified and confirmed DiLiberti et al.’s (1984) description of FVS in 19 children prenatally exposed to VPA. The syndrome becomes apparent as early as at midgestation stage of development (Serville et al., 1989).

Robert and Guibaud (1982) and their colleagues interviewed 146 mothers of infants with spina bifida in the Rhone-Alps region of France and observed an unusual number of them were epileptic and had used VPA during pregnancy. Robert's
pioneering case control studies (Robert, 1982), and the reports of the International Clearinghouse for Birth Defects Monitoring Systems (Bjerkedal et al., 1982), Mastroiacovo et al. (1983) from Italy, and Lindhout and colleagues (Lindhout and Meinardi, 1984; Lindhout et al., 1992)) from The Netherlands provide further evidence for the association of maternal VPA-exposure and fetal spina bifida after making allowance for epilepsy as a confounding factor. These data reveal an absolute risk of 1.2% for spina bifida in the offspring of VPA-treated epileptic women compared with the risk of 0.06% for the general population.

Robert and Rosa (1983) later adduced evidence for other major malformations such as oral clefts and congenital heart malformations. Mastroiacovo et al. (1983) analyzed this data to compare the frequency of oral clefts and cardiac malformations in VPA exposure with that found in infants of mothers who used other AED. They concluded that only the association between maternal VPA use and increased risk for spina bifida persisted even after epilepsy as a confounder was excluded. In other words no data is available to support an argument in favor of specific association of VPA with effects other than spina bifida (Bertollini et al., 1985; Kallen et al., 1989)

What are the risks for malformations other than NTD in valproic acid-exposed pregnancy? Epidemiological studies (see for references Martinez-Frias, 1990) have shown that VPA exposure results in an increased risk for congenital heart diseases but not for oral clefts. Robert and Rosa (1983) observed a total of 9550 malformed infants. The mothers of 91 infants of this study had been exposed to AED; 38 of them had been treated with VPA of which 25 delivered babies with spina bifida, orofacial clefts and heart defects while there were 13 VPA exposed infants among the 7917 infants with other birth defects. They also reported odds ratios of 4.3 for VPA related congenital heart defects and 5.4 for orofacial clefts respectively. Lindhout and Schmidt (1986) considered the combined results of 13 worldwide cohort studies and found 2111 infants exposed to anticonvulsants and observed 12 cases of NTD. They detected a prevalence rate of 2.5% NTD related to VPA monotherapy which was several times higher than that for other AED (0.3%) and for the general population. Dansky and Finnell (1991) compared the results of several published cohort studies and observed, among a total of 153 infants exposed to VPA only, the following malformations in decreasing order of frequency (minor malformations excluded): hypospadias (5), umbilical hernia (4), inguinal hernia (3), spina bifida (3), cardiovascular anomalies (3), skeletal defects (2), and congenital hip dislocation/dysplasia of the hip (2). Curiously among the minor anomalies of phenytoin and phenobarbital exposed infants, nail and phalangeal hypoplasia, hypoplasia of the midface, depressed nose and epicantthal folds disappear at 4 years of age and only the hypertelorism persist, whereas the VPA exposed infants exhibit a characteristic pattern of craniofacial, skeletal and genital minor anomalies that persist; infants with more minor malformations also have major malformations of the skeletal system (Koch et al., 1992).

As Ardinger et al. (1988) rightly pointed out, the major inference from these case reports and case control studies is that "not all children with prenatal VPA exposure have problems that would necessarily draw the attention of a pediatrician in the neonatal period". Also not clear at this moment is the effect of prenatal exposure to VPA on prenatal or postnatal growth. In a prospective cohort study of 11 infants prenatally exposed to VPA, four were found to have FVS and long thin overlapping fingers/toes and hyperconvex nails. Four of them had NTD, and urogenital and cardiovascular defects. Six of these children displayed delay in motor activity and speech development. These are possibly the only studies where growth was carefully monitored (Jager-Roman et al., 1986; 1987). An important strategy in the prevention of VPA- or any other drug-induced congenital malformations is to determine the embryonic mechanisms of dysmorphogenesis. In this regard animal models are of high value because they provide an opportunity to study the effects of suspect teratogens in a situation where the confounding variables could be well controlled.

Experimental studies
Experimental investigations both, predicted and later confirmed the teratogenic properties of VPA (Miyagawa et al., 1971; Whittle, 1976; Brown et al., 1980; Kao et al., 1981; Ong et al., 1983; Brown and Coullouh, 1984; Bruckner et al., 1983; Kelly, 1984; Finnell and Chernoff, 1985; Mast et al., 1985; Nau and Loscher, 1986; Naruse et al., 1988; Paulson et al., 1985; 1989; Petere et al., 1986; Finnell, 1991; Collins et al., 1991; Padmanabhan and Hameed, 1994). Several laboratory animals have been employed in an effort to develop animal models of fetal valproate syndrome so that pathogenetic mechanisms can be investigated. Thus we now have hamster, mouse, rat, rabbit and monkey models (see references above) and in vitro systems (Bruckner et al., 1983; Naruse et al., 1988; Seegmüller et al., 1991; Ehler et al., 1992; Marr et al., 1997) in which the dysmorphogenic potential of VPA can be investigated. Sodium or calcium salts of valproic acid have been administered to pregnant animals in single or multiple doses, often several fold higher than human therapeutic doses during different stages of gestation. Such higher doses were justified in light of the high metabolic rate characteristic of experimental animals and the relatively short half-life of VPA (Nau et al., 1991; Gordan et al., 1995). Nau and Loscher (1986) evaluated the teratogenic potential of the metabolites and several analogues of VPA and discovered that it is the parent compound and not one of its metabolites, which is responsible for the observed teratogenic activity. In addition to maternal toxicity and embryolethality, most commonly craniofacial, skeletal, limb and neural tube malformations (mostly
exencephaly (Padmanabhan and Hameed, 1994) in the MF1 strain and VPA did not significantly alter the background incidence of this defect in the *curly-tail* mouse (Padmanabhan and Hameed, 1994; Padmanabhan and Vaidhya, 1990). Caudal regression characterized by caudal vertebral agenesis/malformation, renal agenesis/hypoplasia and imperforate anus observed in our VPA-treated TO mouse embryos (Padmanabhan and Ahmed, 1996) fit very well with the proposal of Ardingter et al. (1988) regarding a common pathogenetic mechanism for all these anomalies. The difficulty in elucidating this hypothesis further lies in the fact that such caudal defects are only observed in a small number of VPA-treated mouse fetuses. However, a high incidence of exencephaly does occur both *in vivo* and *in vitro* (Bruckner et al., 1983; Nau and Losher, 1986; Naruse et al., 1988; Padmanabhan and Ahmed, 1996). We also recently reported a concordant relationship between NTD and axial skeletal defects in VPA-induced teratogenesis in the *curly tail*, MF1 and TO mice (Padmanabhan and Vaidhya, 1990; Padmanabhan and Hameed, 1994; Padmanabhan and Ahmed, 1996).

**Genetic susceptibility - teratogen interaction.**

The *curly tail* mice have a high incidence of neurovertebral defects including a low incidence (2%) of spina bifida aperta, the TO mice exhibit a 3.5% spontaneous exencephaly whereas the MF1 does not have any background occurrence of NTD at all. GD 7, 8 or 9 (vaginal plug positive day = day 0) were chosen for VPA administration because these are the days shown to be critical for neural tube closure in mice (Copp et al., 1990). Since closure of the neural tube is a delicately balanced multistep process which can be upset by genetic and a variety of environmental factors (Sulik and Sadler, 1993; Seller, 1995) and because the action of neuroteratogens such as alcohol and VPA are suggested to be modified by a mechanism called ‘susceptibility cofactor - teratogen interaction’ (Khoury et al., 1992), we planned to find out if interactions occur in NTD production by VPA.

Single doses of VPA administered on GD 9 caused a fourfold increase in the incidence of NTD (spina bifida) in the *curly tail* mice (Padmanabhan and Vaidhya, 1990). The same dose regimen on GD 9 rescued the embryos of spina bifida. Seller and Perkins (1986) have described similar responses as those due to teratogen-induced growth retardation of the embryos. In the TO mice, VPA was found to significantly augment the spontaneous exencephaly in the GD 7 and GD 8 treatment groups. Female excess in incidence, polyhydramnios, conspicuous absence of the cranial vault, and hemorrhagic degeneration of the exposed brain characterized the abnormality. Exencephalic embryos were markedly growth retarded. In addition to craniofacial and urogenital anomalies, severe axial skeletal malformations were found to be consistently associated with exencephaly.

Although our VPA treated embryos of GD 10 had unclosed
neural folds at site 2, site 3, and sometimes at site 4, most fetuses at term had NTD at site 2 probably because repair had occurred in other locations. The acrania embryos with median facial cleft perhaps represent cases of complete failure of closure at sites 1 through 4. The neural crest cells had failed to migrate and remained stuck to the edges of the unfused neural folds. Vascular lesions, hemorrhage, edema, cell death, failure of neural crest migration, and consequent mesenchymal tissue deficiency might have contributed to closure failure as well as to craniofacial malformations. The retarded migration of neural crest and increased cell death in crest-derived craniofacial tissue, in the optic cup, otic vesicle, and trigeminal and otic ganglia observed in our VPA treated embryos are reminiscent of the description of Seegmiller et al. (1991) in the nasal placode and head mesenchyme of VPA-treated rat embryos in vitro. Histological sections of GD 10 embryos revealed early onset of treatment-related growth inhibition. Arrest of closure appeared to affect intermittent segments of the neural tube. The closure defect sometimes only involved the surface ectoderm of the dorsal midline. The unclosed neural tube was at times found to be covered with a continuous layer of thin surface ectoderm. Cell death per se was not pronounced in the neuroepithelium. The mesenchyme was generally scanty and hemorrhage and edema were obvious in embryos with partial closure. Growth suppression of the optic and otic primordia was marked by pronounced cell death in these structures as well as in the otic and trigeminal ganglia, and in the pharyngeal arch mesenchyme. Clumps of neural crest cells were found stuck along the unfused neural folds. These data indicate that VPA interacts with genetic susceptibility, augments the frequency of exencephaly by arresting the normal closure processes, and also induces other malformations. The widespread malformations of the craniofacial structures are possibly the result of the preferential action of VPA on the neural crest or its derivatives.

PATHOGENETIC MECHANISMS

With regards to VPA-induced malformations, the following cellular and metabolic mechanisms have been suggested:

- reduced intracellular pH (Scott et al., 1997)
- altered embryonic lipid metabolism (Nau et al., 1995)
- altered Zinc metabolism (Nau et al., 1995)
- perturbation of embryonic folate metabolism (Trotz et al., 1987; Padmanabhan, 1997)
- interference with retinoid metabolism (Nau et al., 1995)
- cell death, neural crest cell migration failure, and growth retardation (Padmanabhan, and Hameed, 1994; Padmanabhan and Ahmed, 1996)
- alterations in developmental gene expression (Burns et al., 1996; Finnell et al., 1997; Fatella et al., 2000)

A close look at the literature reveals that these postulates based primarily on experimental data are still largely speculative in humans and credible evidence has not come yet.

While considering the teratogenic mechanisms of VPA, it is important to bear in mind that not all individuals exposed prenatally to this drug are born with malformations and not all features of the FVS are manifest in every affected child. The current estimate is that only 1-2% of all VPA exposed infants will be born with spina bifida. As a unique experiment of nature, twins and triplets exposed to the same maternal blood concentrations of AED express different phenotypes ranging from apparently normal to obvious malformations suggesting that the genetic sensitivity of the embryo is an important factor that determines the outcome (Bustmanns and Stumpff, 1978; Pellock and Nance, 1982; Phelan et al., 1982; Buehler et al., 1990). Obviously the genotype of the embryo, developmental stage at which the exposure occurs, dose and duration of exposure, pharmacokinetics, chemical nature of the drug, and possible teratogen (drug)-susceptibility (gene) interactions need to be considered in determining the mechanisms (Wilson, 1973).

Species differences

The pharmacokinetics and half-life of VPA might be different in different species (0.7 - 2.5 hrs in laboratory animals Vs 8 - 20 hrs in humans) possibly as a result of variations in their ability to metabolize the drug. However, it is not clear why both in humans and experimental animals only a small proportion of all exposed embryos is malformed. The basis of the differential responses may be related to genetic susceptibility of a certain proportion of the population. Thus it can be hypothesized that in each population there are sub-populations of susceptible individuals who carry a certain number of liability genes with which VPA interacts in the production of the congenital malformations (Finnell et al., 1988). The fragility with regards to VPA-induced exencephaly in different strains of mice has been found to exhibit a hierarchy with certain strains being totally resistant, some moderately susceptible while others highly sensitive (Strickler et al., 1985; Finnell et al., 1988; Padmanabhan and Ahmed, 1996). The differential susceptibility may be related to the differences in the ability of the fetuses to produce drug-metabolizing enzymes, which is genetically determined. In fact children with fetal hydantoin syndrome have been shown to have very low levels of epoxide hydrolase activity (Buehler et al., 1990).

Alterations in Folate Metabolism

In view of the fact that VPA is a neuroteratogen in humans and laboratory animals, the role of folates in VPA-induced teratogenesis is of particular interest. The evidence for alterations in folate metabolism contributing to congenital malformations in AED exposed pregnancies comes from the following observations:

Phenytoin and VPA interfere with folate metabolism in the embryo (Netzloff et al., 1979; Hansen and Billings,
Birth defects due to valproic acid exposure

1985; Will et al., 1985)
Folate concentrations decrease during pregnancy and AED including VPA intensify this effect (Hendel et al., 1984)
Epileptic patients with malformed infants have particularly low levels of plasma folate (Dansky et al., 1987; Ogawa et al., 1991)
Homocysteine metabolism is defective in patients with a history of NTD (Steegers-Theunissen et al., 1991)
VPA reduces folate and B12 levels in pregnant mice and high doses of folate augments VPA effects whereas moderate doses partially protects embryos against VPA-induced NTD (Nau et al., 1991; Padmanabhan, 1997, Padmanabhan et al., 1998)
Recent experiments in our laboratory indicate that the dose and time of administration of exogenous folate are crucial in determining the possibility of a rescue effect on embryos from VPA-induced NTD (Padmanabhan, 1997; Padmanabhan et al., 1998). For instance, smaller doses of folic acid (FA) administered over an extended period of time during critical stages of development are found to maintain plasma folate levels higher than that of non supplemented animals and protect the embryonic neural tube against the deleterious effects of VPA. An important issue to address is the mechanism by which FA produces this alleviation effect. Possible interaction between methylene tetrahydrofolate reductase (MTHFR) gene and FA has been implicated in NTD. Thus for example, in the SWV strain highly susceptible to VPA effect, VPA induces a significant reduction in the expression of folate binding protein (FBP-1), while in the more resistant LM/Bc there is an upregulation of this gene and a higher level of expression of MTHFR gene when treated with VPA (Finnell et al., 1997). Since the FA cycle involves a number of other micronutrients such as B12, methionine, homocysteine, betaine etc., consideration of FA in isolation may not reveal the whole story. Recent data from our laboratory show that methionine is not helpful in preventing ethanol-induced exencephaly in mice but addition of homocysteine during critical period of neural tube development has profound rescue effect on VPA-induced exencephaly (Padmanabhan et al., 2000 unpublished data).

SUMMARY AND CONCLUSIONS
With maternal epilepsy per se contributing to congenital malformations similar to those induced by AED, it would appear that various AED only augment epilepsy-associated birth defects. The mechanisms need to be investigated. VPA induces spina bifida in humans and exencephaly in laboratory animals. Since NTD are known to be alleviated substantially by supplemental folic acid (FA), it would be beneficial to know if FA would also prevent NTD in maternal exposure to VPA.

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