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Developmental cardiotoxicity effects of four commonly used antiepileptic drugs in embryonic chick heart micromass culture and embryonic stem cell culture systems

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ABSTRACT

Antiepileptic drugs (AEDs) are commonly used drugs in pregnant women with epilepsy. Prenatal exposure to AEDs increases the risk of major or minor congenital malformation during embryonic development. The precise mode of action and intracellular mechanisms of these AEDs during embryonic development remains unclear. To determine relative teratogenic risk of AEDs, four AED drugs including valproic acid (VPA), phenytoin (PHT), phenobarbital (PB), and trimethadione (TMD) were tested using two *in vitro* systems (the embryonic chick heart micromass (MM) culture and the *in vitro* differentiating mouse embryonic stem cells into cardiomyocytes culture systems). Cardiomyocyte cultures (the heart MM and ES cell-derived cardiomyocytes) were treated with or without different concentrations of PHT, PB, TMD (10–100 μM), and VPA (100–2000 μM). 5-Fluorouracil (5-FU) (1–10 μM) and L-ascorbic acid (10–1000 μM) were used as positive and negative controls. It was found that these four commonly used AEDs and 5-FU tested have the potential to inhibit embryonic heart cell differentiation ($p < 0.05$) without inducing any cytotoxicity. VPA at higher concentrations ($\geqslant$800 μM), and 5-FU at all doses proved to be cytotoxic in the differentiating ES cell culture system. The results demonstrated in this study suggest that the use of these four commonly prescribed AEDs during pregnancy may have an effect on embryonic heart development, and may be associated with congenital cardiovascular defects.

1. Introduction

Antiepileptic drugs (AEDs) are commonly prescribed drugs to women with epilepsy during pregnancy. The precise mode of action and intracellular mechanisms of these drugs to embryonic cardiac development remain unclear (Perucca, 2005). However, maternal exposure to AEDs in the first trimester of pregnancy has been associated with an increased risk of major and minor congenital anomalies in the offspring (Perucca et al., 2006). Animal studies have shown that *in utero* AED exposure can produce anatomical, behavioral and developmental defects which can occur in response to concentrations of AEDs lower than those required to produce congenital malformation (Meador et al., 2006). Although, the AEDs are important during pregnancy in epileptic women to avoid complication, women who stop taking AEDs are at more risk of developing status epilepticus, which has a high mortality rate for mother and baby. Convulsive seizures in pregnancy can cause fetal intracranial haemorrhage and heart rate changes (Fountain, 2009), thus maintaining a serum therapeutic level of AEDs during pregnancy is necessary to avoid maternal and fetal life risks (Ornoy, 2006; Pennell, 2005).

This article presents four commonly prescribed AEDs including valproic acid (VPA), phenytoin (PHT), phenobarbital (PB) and trimethadione (TMD) that are taken by epileptic women during pregnancy and may have an adverse effect on the fetus.

1.1. Valproic acid (VPA)

VPA was first discovered as an anticonvulsant in 1974. Since then, it has been used in many countries because of its antiepileptic properties to treat several types of epilepsy as well as being used as a mood stabilizer (Ornoy, 2006). VPA has also been used to treat several diseases including psychological disorders such as schizophrenia, borderline personality disorder, alcohol, cocaine withdrawal and dependence, as well as neurological disorders (i.e. Alzheimer's Disease (AD), and tardive dyskinesia) (Peterson and Naunton, 2005). The precise mechanisms by which VPA exerts

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its antiepileptic effects remain to be conclusively determined. Most of the anticonvulsant drugs are teratogenic in humans as well as in experimental animal models (Schilter et al., 1995). VPA has the widest use of all AEDs, and is given to relieve symptoms either in mono or poly-therapy, as previously mentioned (Crawford et al., 1999; Peterson and Naunton, 2005). Although it has been considered to be safe for epileptic pregnant women, VPA exposure has been associated with certain birth defects such as spina bifida, congenital anomalies of the heart and craniofacial features, neural tube defects (NTDs), urogenital and limb defects (Finnell et al., 2002; Isoherranen et al., 2003; Whitsel et al., 2002). It has been shown that the levels of VPA used to closely mimic the *in vivo* situation strongly elevate intracellular reactive oxygen species (ROS) that are in part responsible for the inhibition of cardiomyogenesis *in vitro* (Na et al., 2003). Furthermore, the concentration of VPA in the blood plasma of pregnant women could reach as high as 1162 μM (see Table 1 the therapeutic blood plasma level of VPA). This corresponds to the maximum concentration of VPA (1000 μM) used in the study of Na et al., 2003, approximately this concentration of VPA, produce a condition known as 'valproate embryopathy' and this causes several congenital anomalies including tall forehead, depressed nasal bridge, hypertelorism, epicanthic folds, long smooth philtrum, small mouth with thin upper lip and full lower lip, posteriorly rotated ears, and overlapped toes (Schorry et al., 2005).

1.2. Phenytoin (PHT)

PHT is the most widely used AED, and is generally considered to be very effective in controlling most forms of epilepsy (Yaari et al., 1986). The average dose of PHT in adults is 5 mg/kg daily and can be given either orally or by injection to treat most forms of epilepsy (Malone and D'Alton, 1997). Additionally, PHT has been applied therapeutically in the treatment of arrhythmias because of its pharmacological and electrophysiological properties of stabilizing membranes of excitable cells, including neuronal and cardiac muscle cells (zur Nieden et al., 2004) (see Table 1 the therapeutic blood plasma level of PHT). It can decrease resting fluxes of Na^+, as well as active Na^+ currents that flow during action potentials or chemically induced depolarization (Jones et al., 1983). PHT has been found to be teratogenic in the humans (Adams et al., 1990). The use of PHT during pregnancy has been associated with embryological malformation and developmental defects. In addition PHT use is associated with the pattern of a distinct malformation syndrome as a result of PHT exposure during pregnancy, that is more commonly described as the Fetal Hydantoin Syndrome (FHS) (Ornoy, 2006). The FHS has been characteristic by dysmorphic features including short nose, flat nasal bridge, up-turned nasal tip, wide lips, hypertelorism, epicanthal folds, ptosis, low set ears, low hairline, short neck, distal digital hypoplasia, absent nails, altered palmer crease, digital thumb, and dislocated hip (Malone and D'Alton, 1997). The teratogenicity of PHT has been demonstrated in different species such as mice, rats, rabbits and cats. The defects include major structural abnormalities such as orofacial clefts, limb, urogenital, and cardiovascular defects, as well as specific minor congenital malformations, e.g. distal digital defects, midfacial hypoplasia (broad depressed nasal bridge, short upturned nose), growth retardation, and developmental delay/mental retardation (Azarbayjani and Danielsson, 1998).

1.3. Phenobarbital (PB)

Phenobarbital (PB) is a sedative hypnotic barbiturate drug that can also be used as an anticonvulsant. It is widely used to treat partial and generalized tonic-clonic seizures and epilepsy (Malone and D'Alton, 1997). It is used either orally or by injection. The average dose in adults is 60–240 mg/day. The lowest dose is used as a starting dose to minimize sedating side effects. This concentration is used to achieve steady state plasma PB concentration within the range of 40–130 μM (see Table 1 the therapeutic blood plasma level of PB) (Danielsson et al., 2007; Finnell et al., 1987). PB acts pharmacologically by blocking the voltage dependant Ca^{2+} channels in excitable cells like neurons and cardiac muscle cells. Previously it has been shown that PB can alter the basic mediators of neuronal cell excitability by inhibition/blockage of depolarization stimulated Ca^{2+} channels influx into synapse of neurons, thus resulting in reduced depolarization and neurotransmitter release in various systems (Leslie et al., 1980). Maternal exposure to PB has also been associated with the same major and minor embryological malformations and dysmorphic developmental features as with PHT. These include major (orofacial clefts and congenital heart defects) and minor (digital, craniofacial and growth retardation) congenital malformations (Azarbayjani and Danielsson, 1998; Holmes et al., 2004).

1.4. Trimethadione (TMD)

TMD (3,5,5-trimethyl-2,4-oxazolidinedione) is an anticonvulsant drug used in the treatment of petit-mal epilepsy (Malone and D'alton, 1997). It is used either orally or by injection (see Table 1 for therapeutic blood plasma concentration of TMD in humans). TMD is rapidly metabolized by N-demethylation to the pharmacologically active metabolite dimethadione (DMD) which is not known to metabolized any further. TMD and DMD were both

Table 1
Human blood plasma therapeutic concentration level of four commonly prescribed AEDs and drug concentration used in the screening part of this embryotoxicity/teratogenicity study with the two *in vitro* systems (the embryonic chick Heart MM culture and ES cell-derived cardiomyocytes culture systems).

Drugs	Treatment classes	Therapeutic plasma concentration (μM)	Drug concentration in assay (μM)	Major congenital anomalies reported
Valproic acid (VPA)	Anticonvulsive	350–1162[a,b,c]	100–2000	Congenital heart, limb, neural tube, urogenital, craniofacial and growth retardation
Phenytoin (PHT)	Anticonvulsive	40–80[d,f]	10–100	Congenital heart, limb, cleft palate (craniofacial), urogenital and growth retardation
Phenobarbital (PB)	Anticonvulsive	40–130[d,e]	10–100	Craniofacial, cardiovascular, urogenital, and central nervous systems defects
Trimethadione (TMD)	Anticonvulsive	3000–10,000[d,f]	10–100	Intrauterine growth retardation, cardiovascular, craniofacial, limb and urogenital defects

[a] (Ornoy, 2006).
[b] (L'Huillier et al., 2002).
[c] (Na et al., 2003).
[d] (Danielsson et al., 2007).
[e] (Finnell et al., 1987).
[f] (Azarbayjani and Danielsson, 1998).