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Teratogenicity of Valproic Acid: Can Reverse its Possible Effects on Testicular Development with Vitamin E?

Teratogenicidad del Ácido Valproico: ¿Se Pueden Revertir sus Posibles Efectos sobre el Desarrollo Testicular con la Vitamina E?

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ABSTRACT: Epilepsy is the chronic non-communicable disease of the nervous system most prevalent in the world. Valproic acid (VPA) is one of the most used drugs in the treatment of epilepsy but with various side effects. One of the organs that can be affected is the testis, where it has been seen that men treated with VPA reduce their fertility rates, in addition to causing endocrine disorders by decreasing androgens and gonadotropins. In animal models, it has been shown to reduce the weights of the glands attached to the male reproductive tract, as well as at the testicular level, decreasing sperm concentration and increasing apoptotic cell count. These effects are because VPA increases reactive oxygen species (ROS), causing damage to macromolecules and affecting all cellular processes sensitive to oxide reduction. Throughout testicular development, in utero, it has been seen that the expression of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, are lower during early embryonic development, as well as vitamin E (VE) is decreased. Therefore, they are not sufficient to reverse the toxic effects of ROS. The objective of this study was to review the use of VPA during pregnancy, its effect on testicular development, and to explore the potential protective role of vitamin E.

KEY WORDS: Epilepsy; Pregnancy; Valproic acid; Vitamin E; Testicular development.

INTRODUCTION

Epilepsy is a neurological disorder affecting approximately 50 million people worldwide, making it the most prevalent chronic non-communicable neurological disease. About 80 % of these individuals reside in low- and middle-income countries. Each year, an estimated 2.4 million people are diagnosed with epilepsy globally (World Health Organization, 2024).

Epilepsy is characterized by recurrent, unprovoked seizures, which can manifest as focal-onset, generalized, or of unknown origin. The disorder's symptoms vary depending on the specific brain regions affected and the spread of neuronal electrical discharges (Beghi *et al*., 2015; Scheffer *et al*., 2017). Epilepsy is associated with physical complications such as fractures and bruises resulting from seizures, as well as psychosocial disorders including anxiety and depression. People with epilepsy face a three-fold increased risk of premature death compared to the general population (World Health Organization, 2024).

During pregnancy, the use of anticonvulsants like valproic acid (VPA) increases the risk of congenital malformations in various fetal organs (Ornoy *et al*., 2018). This review aims to explore the impact of VPA use during pregnancy specifically on testicular development, while also examining the potential protective role of vitamin E (VE).

Epilepsy and Pregnancy. Epilepsy is highly prevalent among women of reproductive age, with potential implications for pregnancy (Viale *et al*., 2015). In North America, approximately 7 % of individuals with epilepsy are pregnant at any given time (Tomson & Battino, 2009).

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Generalized tonic-clonic or non-convulsive seizures during pregnancy can trigger several adverse events, including increased blood pressure, lactic acid production, hypoventilation, elevated intra-abdominal pressure, redistribution of cerebral blood flow, prolonged uterine contractions, fetal distress, and stillbirth (Kashif *et al*., 2019). Moreover, the risk of complications such as metrorrhagia, hyperemesis gravidarum, anemia, and eclampsia is heightened, often doubling the likelihood of requiring cesarean section. The act of childbirth itself poses additional risks, as it increases the likelihood of seizures and is associated with hypoxia, acidosis, fetal bradycardia, acute fetal distress, and perinatal death (Viale *et al*., 2015).

In managing epilepsy, anticonvulsants play a crucial role against this backdrop. Among the first-line medications are carbamazepine, phenytoin, and valproic acid (VPA), typically used as monotherapy at minimal effective doses to control the condition (Kashif *et al*., 2019). During pregnancy, epileptic women experience physiological changes such as increased renal clearance, expanded blood volume, induction of hepatic P-450 enzymes, reduced gastrointestinal absorption, heightened emotional stress, and disrupted sleep patterns. These factors collectively lower plasma levels of antiepileptic drugs, heightening the risk of seizures (Harden, 2014).

Among the drugs mentioned, valproic acid (VPA) stands out as one of the most prescribed due to its high efficacy. This is attributed to its ability to increase gamma-aminobutyric acid (GABA) levels in the brain, which, when deficient, can lead to seizures (Morland *et al*., 2012). Other proposed mechanisms of action include the inhibition of voltage-gated sodium channels, suppression of neuronal metabolism, and reduction of excitatory aspartate neurotransmission (Ornoy, 2009). Consequently, VPA is also utilized in the treatment of mood disorders, bipolar disorders, and headaches (Khan *et al*., 2016).

Valproic Acid and Alterations of Embryo-Fetal Development. During pregnancy, the use of antiepileptic drugs poses a significant risk to embryo and fetal development, as studies have established a causal relationship between their use and an increased incidence of congenital malformations, with the risk ranging from 5 % to 9 % (Ahmed *et al*., 2014). Valproic acid (VPA) is considered among the most teratogenic of these drugs, with estimates suggesting a risk 2 to 7 times greater compared to other commonly used antiepileptic medications. The International League Against Epilepsy (ILAE) recommends avoiding VPA when feasible, opting instead for alternative drugs with lower risks of malformation (Tomson *et al*., 2019). However, for pregnant women who require continued VPA treatment to manage their condition effectively, it is advised not to discontinue its use without proper medical guidance (Tung & Winn, 2011a).

VPA increases the risk of various congenital malformations including neural tube defects, congenital heart defects, genitourinary abnormalities, musculoskeletal abnormalities, and cleft lip or palate (Tanoshima *et al*., 2015). Specifically, regarding genitourinary defects, a case-control study has demonstrated that VPA monotherapy carries a higher risk of genital congenital malformations compared to carbamazepine, lamotrigine, and combination therapies (Veiby *et al*., 2014). Cryptorchidism and hypospadias are notably prevalent, often associated with fetal valproic acid syndrome (Laganà *et al*., 2016; Mutlu-Albayrak *et al*., 2017). VPA crosses the placenta readily, resulting in higher serum concentrations in the umbilical cord compared to maternal serum, with levels up to five times greater by the end of pregnancy (Omtzigt *et al*., 1992).

One proposed mechanism of teratogenicity involves the metabolism of folic acid, where reduced levels during embryonic stages, alongside increased oxidative stress, can disrupt protein synthesis crucial for prenatal development (Wegner & Nau, 1992). This mechanism is associated with neural tube closure defects, cardiac malformations, and neurodevelopmental alterations induced by valproic acid (VPA) exposure (Alsdorf & Wyszynski, 2005). However, findings from human studies and animal models present contradictory evidence. Human studies indicate that folic acid supplementation in pregnant women exposed to VPA during the first trimester does not significantly reduce the risk of major congenital malformations (Morrow *et al*., 2009; Jentink *et al*., 2010). Conversely, animal models demonstrate that VPA alters folate metabolism in mouse embryos, leading to elevated levels of tetrahydrofolate and reduced levels of 5-formyl- and 10-formyl-tetrahydrofolates (Wegner & Nau, 1992; Shona *et al*., 2018). Nevertheless, consensus across studies highlights the need for further research to fully elucidate these implications.

Moreover, murine models of autism induced by VPA have shown increased oxidative stress during prenatal development, characterized by elevated levels of nitric oxide, lipid and protein peroxidation, and reduced enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione (Al-Amin *et al*., 2015). In embryonic cell cultures, VPA has been demonstrated to increase reactive oxygen species (ROS) and apoptosis. The addition of catalase to the medium mitigated the VPA-induced rise in ROS formation and apoptosis, underscoring the role of oxidative stress in VPA's teratogenic effects (Tung & Winn, 2011b). These findings highlight the susceptibility of embryonic

development to VPA-induced alterations in oxidative stress, suggesting a potential molecular pathway through which VPA exerts its teratogenicity.

Another proposed mechanism involves the inhibition of histone deacetylases (HDACs), enzymes responsible for reducing histone acetylation levels, thereby influencing chromatin structure and impacting the activity of transcription factors and RNA polymerase involved in gene transcription modulation. HDAC inhibitors like VPA can disrupt the cell cycle, induce growth arrest, and promote apoptosis. This mechanism is exemplified by its effects on pancreatic and cardiac organogenesis (Komariah *et al*., 2018; Philbrook *et al*., 2019). Thus, VPA's impact on HDACs provides a plausible explanation for its teratogenic effects.

Testicular Development. Sexual differentiation in mammals involves a complex series of sequential processes, guided by genetic information from sex chromosomes. Initially bipotential, the gonad differentiates morphologically into ovaries or testes (O'Shaughnessy & Fowler, 2014). Numerous nuclear and transcription factors, along with signaling proteins, play crucial roles in this process. The SRY gene serves as the primary regulator of sex determination. In XY embryos, SRY expression begins at 10 days post-coitum (dpc) in mice and at approximately 42 days in humans, initiating the differentiation of pre-sustentacular or pre-Sertoli cells (Lucas-Herald & Bashamboo, 2014). This early development in male gonads leads to the formation of testicular cords, observed around 13.5 dpc in mice, where sustentacular cells envelop germ cells, forming seminiferous cords surrounded by peritubular myoid cells (Huhtaniemi & Pelliniemi, 1992) (Fig. 1).

During embryonic development, energy in the form of ATP, NADPH, and oxygen is essential for proper growth and differentiation. These compounds are generated through the mitochondrial electron transport chain, a tightly regulated process (Dennery, 2004). The uterine environment is typically hypoxic, making organogenesis sensitive to oxidative stress. As uteroplacental circulation is established and the placenta becomes functional for nutrient and gas exchange, the embryo's antioxidant defenses improve, enhancing its ability to withstand oxidative stress (Burton, 2009). Oxidative levels, and the programmed changes around them known as redox switching, influence cell fate decisions in the embryo ranging from proliferation and differentiation to apoptosis or necrosis. Reduced states favor proliferation, mild oxidation promotes differentiation, and higher oxidation levels can induce cell death (Schafer & Buettner, 2001).

Disruption of the delicate balance of oxidative stress can lead to abnormalities affecting germ cells, gonadal development, embryos, and fetuses, with potential long-term consequences for the mature organism, depending on the timing of these events (Dennery, 2004). Germ cells are particularly sensitive to oxidative stress due to the high concentration of polyunsaturated fatty acids in sperm cells, making them highly susceptible to reactive oxygen species (ROS). Sperm cells themselves generate low levels of hydrogen peroxide and superoxide, crucial for the capacitation process needed for sperm penetration of the oocyte's zona pellucida (Kim & Parthasarathy, 1998). Spermatozoa have limited defenses against oxidative stress, leaving them vulnerable to oxidative DNA damage, affecting both mitochondrial and nuclear levels, thereby

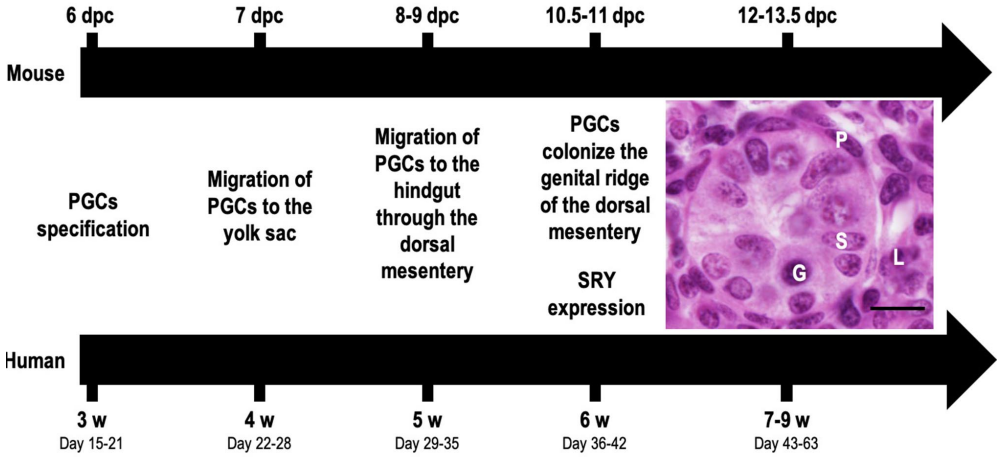

Fig. 1. Timeline of early testicular development in humans and mice. Days post-coitum (dpc); Weeks (w); Primordial germ cells (PGCs); Gonocyte (G); Sustentacular cell (S); Peritubular cell (P); Interstitial cell (L). At 13.5 dpc in mice and between 7 to 9 weeks in humans, the formation of testicular cords is evidenced, where support cells (S) and gonocytes (G) form testicular cords surrounded by peritubular cells (P). In the interstitial spaces, the interstitial cells (L) are found. Bar 20 μm.

impairing mitochondrial biogenesis and altering protein synthesis (Baker & Aitken, 2005; Aitken & Baker, 2006).

In spermatogenesis, the rapid cell division inherent in this process increases mitochondrial oxygen consumption by the germinal epithelium. The testes, with their poor vascularization, maintain low oxygen concentrations, intensifying competition for this vital element and impacting steroidogenesis (Peltola *et al*., 1994). Furthermore, the presence of highly unsaturated fatty acids in sperm and ROS-generating systems from mitochondria, enzymes such as xanthines, NADPH-oxidases, and cytochrome P-450, contribute to the production of toxic metabolites (Kumagai *et al*., 2002; Chen *et al*., 2005).

Valproic Acid and Alterations in Testicular Development. Despite the antioxidant defenses present in the testes to support their dual functions of steroidogenesis and spermatogenesis, numerous endogenous and exogenous factors have been studied that disrupt these defenses and induce oxidative stress, potentially leading to alterations in testicular development (Aprioku, 2013). Men with epilepsy often experience reduced fertility rates, accompanied by endocrine disorders and sexual dysfunction (Hamed, 2016). VPA has been specifically linked to endocrine disruptions, including decreased serum concentrations of androgens and gonadotropins in men with epilepsy (Rättyä *et al*., 2001). Infertility cases have also been reported among male subjects undergoing VPA treatment (Yerby & McCoy, 1999). *In vitro* studies have indicated that VPA may have more detrimental effects on sperm motility compared to other antiepileptic drugs such as carbamazepine, phenobarbital, or phenytoin (Chen *et al*., 1992).

The results of studies conducted in humans or animal models, predominantly rats or mice, are summarized in Table I, revealing common morphological alterations such as decreased volumes and weights of the testes, prostate, and seminal vesicles, along with endocrine and spermatic abnormalities. Among these, a noteworthy study conducted in Wistar rats in 2011 stands out as the only one to date evaluating testicular development in a group treated with VPA. This study determined that in the adult stage, the relative weights of the testes were significantly lower in the VPA-treated group compared to the control group. Moreover, there was a decrease in the numbers of spermatogonia, spermatocytes in pachytene, and round spermatocytes at all stages. Apoptotic cell counts and p53 immunoreactivity were notably elevated, while TGF-β1 expression was lower in the VPA group compared to the control. These findings demonstrate that VPA treatment negatively affects spermatogenesis not only by reducing testicular weight but also by increasing apoptotic cell death and p53 expression, and decreasing TGF-β1 levels (Cansu *et al*., 2011).

Table I. Studies in humans or animal models that relate the use of valproic acid and testicular alterations.

Author	Year of publication	Model	Dose of valproic acid	Outcomes
Nishimura *et al*.	2000	Rat	500 mg/kg/day	Sequential damage with decreased epididymal mass, seminal vesicles and prostate, reduced sperm count and sperm motility, degeneration of seminiferous tubules.
Rättyä *et al*.	2001	Human	1,219±413 mg/day	Association with endocrine disorders, decreasing serum concentrations of androgens and gonadotrophins in patients with epilepsy
Sveberg Røste *et al*.	2001	Rat	100-400 mg/kg/day	Decreased testicular mass and testicular atrophy with spermatogenic arrest, being proportional to the dose of VPA.
Isojärvi *et al*.	2004	Human	1,079±412 mg/day	In monotherapy and polytherapy produced high levels of androstenedione, altered sperm parameters and a lower testicular volume.
Bauer *et al*.	2004	Human	1,085±527 mg/day	Monotherapy decreased levels of LH, FSH, testosterone and inhibin B.
Cansu *et al*.	2011	Rat	300 mg/kg/day	Lower testicular mass was observed, germinative epithelium decreased at all stages, apoptotic cell counts and p53 were high and TGF-β1 expression was lower.
Khan *et al*.	2011	Mouse	100-600 mg/kg/day	Sperm DNA damage and decreased expression of 8-oxo-dG at the testicular level.
Hamed *et al*.	2015	Human	750.7±100.45 mg/day	Decreased freet estosterone and testicular volume and altered sperm parameters.

Vitamin E as a protective agent against the damage caused by valproic acid at the testicular level?. Oxidative stress is a major contributor to the alterations associated with the use of VPA, primarily due to excessive production of reactive oxygen species (ROS). These ROS can directly damage macromolecules such as DNA, proteins, and lipids, thereby disrupting cellular signaling pathways. This oxidative stress activates transcription factors sensitive to redox changes, thereby affecting all cellular processes involved in testicular development (Sha & Winn, 2010; Yoon *et al*., 2014). Studies have observed a reduction in glutathione peroxidase levels in the testes of rats treated with VPA, underscoring the impact of VPA-induced oxidative stress (Khan *et al*., 2011). Chronic use of VPA is also known to decrease levels of vitamin E (VE) and glutathione peroxidase, further exacerbating oxidative stress (Al Deeb *et al*., 2000).

The detoxification of ROS involves both enzymatic and non-enzymatic antioxidant mechanisms (Kase *et al*., 2012). Despite the embryo possessing enzymatic antioxidant defenses, these mechanisms are often insufficient to fully mitigate the toxic effects of ROS (Ornoy, 2007). Therefore, antioxidants have been explored as a potential strategy to counteract the damaging effects of free radicals. Antioxidants have shown promise in inhibiting lipid peroxidation, reducing ROS generation, preventing apoptosis, preserving mitochondrial function, protecting cell membranes from cytotoxic damage, and reducing oxidative damage to proteins and DNA (Cárdenas-Rodríguez *et al*., 2013).

α-tocopherol (VE), a fat-soluble vitamin and potent antioxidant, is depleted in both epilepsy and VPA treatment. VE functions by inhibiting the propagation of radical chain reactions within the lipid components of cell membranes. It can penetrate the blood-brain barrier, accumulating to therapeutic levels in the central nervous system, where it reduces markers of oxidative stress (Traber & Atkinson, 2007). Due to its effectiveness as a free radical scavenger in the brain, VE has garnered attention for its neuroprotective role in various neurological disorders (Brigelius-Flohé, 2009). By regulating reactive oxygen species (ROS) production and maintaining oxidative phosphorylation in mitochondria, thereby enhancing high-energy phosphate replacement, VE acts as a potent antioxidant (Kotegawa *et al*., 1993). Studies have shown significantly decreased VE concentrations in patients with uncontrolled epilepsy compared to seizure-free and controlled epileptic groups (Mehvari *et al*., 2016). Additionally, VE has been reported to prevent iron-induced seizures and delay the onset of intracerebral ferrous chloride injection-induced EEG seizures (Pagni & Zenga, 2005). Therefore, supplementation with VE may offer a rational and complementary approach for treating epilepsy.

Studies in animal models have demonstrated that the adverse effects of VPA can potentially be mitigated by prior administration of VE (Zhang *et al*., 2010; Hsieh *et al*., 2014). Additionally, combined administration of folic acid and VE has been shown to reduce the detrimental effects of VPA on the sciatic nerve in rats (Aluclu *et al*., 2009). VE has also exhibited a protective role in neural tube development in VPA-treated mouse embryos (Conei *et al*., 2016). At the testicular level, Ourique *et al*. (2016) conducted a study in Wistar rats to evaluate VE's protective effects against functional abnormalities induced by oxidative stress in the male reproductive system due to VPA. They found that VE restored antioxidant potential, prevented oxidative damage in the testes and epididymis, and restored sperm motility. This suggests that VE could be beneficial in minimizing reproductive impairment in patients requiring VPA treatment (Table II). To date, no studies have investigated the use of VE during pregnancy to mitigate the effects of intrauterine VPA exposure.

CONCLUSIONS

Epilepsy, one of the most prevalent chronic diseases among women of reproductive age, necessitates careful

Table II. Studies where vitamin E is evaluated as a protective agent against the damage caused by valproic acid.

Author	Year of publication	Model	Dose of vitamin E	Outcomes
Al Deeb *et al*.	2000	Mouse	250 mg/kg	Decrease in neural tube defects caused by valproic acid.
Aluclu *et al*.	2009	Rat	250 mg/kg	Co-administration with folic acid (400 mg / kg) decreases the deleterious effects of VPA in ischial nerve.
Zhang *et al*.	2010	Mouse	50 mg/kg	Regulation of the levels of total glutathione in its reduced and oxidized forms, and the expression of Hox genes, decreasing the defects in the development of neural tube, musculoskeletal and yolk sac circulation.
Hsieh *et al*.	2014	Chicken	60 mmol/L	Decrease in neural tube defects caused by valproic acid.
Conei *et al*.	2016	Mouse	200 UI/kg	Protective role at the level of the neural tube and spinal cord of fetuses treated with VPA, regulating the expression of Shh.

management due to potential risks during pregnancy and the adverse effects of antiepileptic treatments on the fetus, notably VPA. Numerous studies in both human and animal models have documented that VPA induces endocrine disorders and morphological abnormalities in the male reproductive system, including reduced weight of the testes, epididymis, seminal vesicles, and prostate, along with damage to seminiferous tubules and sperm.

The damage caused by excessive production of ROS alters macromolecules and disrupts gonadal development. Vitamin E has been shown to inhibit the propagation of radical chain reactions in the lipid portion of cell membranes, and levels of VE are decreased in epileptic patients. Therefore, supplementation with VE could potentially mitigate the alterations caused by valproic acid in the development of various organs. While a single study has assessed the effects of VPA on testicular development, showing decreases in testicular weights and sperm parameters alongside increased apoptotic counts, no studies have reported on the combined effects of VPA and VE on testicular development, warranting further investigation into VE's potential protective effects on this organ.

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RESUMEN: La epilepsia es una enfermedad crónica no transmisible que afecta al sistema nervioso más prevalente en el mundo. Dentro de los tratamientos, uno de los fármacos más utilizados es el ácido valproico (AVP), el que ocasiona diversos efectos secundarios. Entre los órganos que se pueden ver afectados se encuentra la gónada masculina, en donde se ha visto que hombres en tratamiento con AVP reducen sus tasas de fecundidad, además de causar trastornos endocrinos disminuyendo andrógenos y gonadotrofinas. En modelos animales, se ha visto que disminuye los pesos de las glándulas anexas al tracto reproductor masculino, como también a nivel testicular, disminuyendo la concentración espermática y aumentando el recuento de células apoptóticas. Estos efectos se deberían a que el AVP aumenta las especies reactivas de oxígeno (ROS), ocasionando daño en macromoléculas, afectando todos los procesos celulares sensibles a óxido reducción. A lo largo del desarrollo testicular, in utero se ha visto que la expresión de enzimas antioxidantes como superóxido dismutasa, catalasa y glutatión peroxidasa, son más bajos durante el desarrollo embrionario temprano, como también la vitamina E (VE) se encuentra disminuida. Por tanto, no resultan suficientes para revertir los efectos tóxicos de las ROS. El objetivo de esta revisión fue asociar el uso de AVP durante la gestación y sus efectos a nivel del desarrollo testicular y describir el potencial rol protector de la VE.

PALABRAS CLAVE: Epilepsia; Gestación; Ácido valproico; Vitamina E; Desarrollo testicular.

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