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Molecular Pathogenesis of Valproate-Induced Neural Tube Defects: A Systematic Review of Apoptosis, Folate Pathways, and Gene Expression Dysregulation

Background: Significant morbidity and mortality are related to neural tube defects (NTDs), which result from failure of neural tube closure during early development. Through several teratogenic processes, valproic acid (VPA), a commonly used antiepileptic treatment, is strongly correlated with a higher incidence of NTDs.

Objective: This review attempts to synthesize current research and integrate molecular mechanisms associated with VPA-induced NTDs, which are often studied separately.

Methods: Based on PRISMA 2020 guidelines, this study was conducted, and the protocol was registered in PROSPERO (CRD420261384605). Literature from PubMed, ProQuest, ScienceDirect, and Wiley were screened independently by all authors according to inclusion and exclusion criteria, yielding seven studies for this systematic review. Risk of Bias assessment was conducted using the SYRCLE and Modified SYRCLE's RoB.

Results: The results showed that VPA causes NTDs via a variety of pathways, including oxidative stress, apoptosis, folate-pathway dysregulation, epigenetic modification, and retinoic acid signaling disruption. Genetic susceptibility influenced teratogenic outcomes through differential regulation of folate-related and anti-apoptotic genes, whereas VPA exposure changed the expression of genes involved in neurulation and embryonic development. Additionally, recent studies indicate that by inhibiting apoptotic pathways and reducing the prevalence of NTDs, maternal immune activation can reduce the teratogenic effects of VPA.

Conclusion: Developmental signaling dysregulation and host susceptibility factors interact in a complex manner in VPA-associated teratogenicity. To support better preventive and therapeutic strategies for pregnant individuals in need of VPA treatment, further mechanistic research is required.

Keywords: Valproic Acid, Neural Tube Defects, Folate metabolism, Apoptosis, Epigenetic regulation

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Introduction

Neural Tube Defects (NTDs) occur as a result of failure in neural tube closure during embryogenesis. They are considered one of the most severe congenital disorders affecting the central nervous system. These defects arise from abnormal embryogenesis affecting the central nervous system.[1,2] In vertebrates, the central nervous system is formed through neurulation during embryogenesis, a process involving the formation of the neural tube, which later develops into the brain and spinal cord. This process occurs approximately between days 17 and 28 after fertilization.[1] The neural tube is formed when the neural plate undergoes shaping, bending, and fusion during neurulation. Closure of the neural tube occurs through fusion at the dorsal midline, resulting in the formation of a complete neural tube. Failure of this closure process may lead to neuronal degeneration and developmental abnormalities due to exposure of the neuroepithelium to the external environment. Spina bifida and anencephaly are the most common forms of NTDs.[2] Globally, Neural Tube Defects (NTDs) are among the most common congenital abnormalities, with prevalence rates ranging from 0.5 to more than 10 cases per 1,000 pregnancies, particularly in low- and middle-income countries.[1,3] Failure of neural tube closure can result in permanent neurological impairment, neuronal degeneration, motor and sensory deficits, long-term disability, and even neonatal death in severe cases, thereby contributing significantly to the morbidity and mortality associated with NTDs.[1,2,4] Neural Tube Defects (NTDs) are multifactorial disorders involving both environmental and genetic factors, including exposure to teratogenic drugs such as valproic acid.

Valproic acid (VPA) is a medication commonly used to control seizures, stabilize mood, and prevent recurrent migraine attacks. However, the use of valproic acid during pregnancy may be harmful due to its high teratogenic potential. Several studies have shown that valproic acid (VPA) is one of the antiepileptic drugs associated with the greatest teratogenic risk and is linked to various major congenital malformations in the fetus. The most commonly reported abnormalities include neural tube defects (NTDs), congenital cardiac abnormalities, skeletal malformations, and orofacial clefts. One of the mechanisms contributing to the occurrence of neural tube defects is disruption of folate metabolism. Valproic acid (VPA) may interfere with the absorption and metabolism of folic acid, and such folate disruption can inhibit neural tube closure during embryogenesis. In addition to affecting folate metabolism, valproic acid (VPA) also acts as a Histone Deacetylase Inhibitor (HDACi). HDAC inhibition may alter chromatin structure and affect gene expression during embryonic development. These alterations in gene expression can disrupt neural cell proliferation, differentiation, and development, resulting in abnormal neural tube formation and closure.[5] Valproic acid (VPA) has been reported to interfere with neural tube development during embryogenesis. However, despite the well-established association between valproic acid and neural tube defects, the underlying mechanisms responsible for VPA-induced NTDs remain incompletely understood.

Several studies have reported that embryonic exposure to valproic acid affects multiple biological processes involved in neurulation and neural tube closure. Commonly proposed mechanisms include disruption of folate metabolism, increased oxidative stress, histone deacetylase inhibition, altered DNA methylation, dysregulation of gene expression, impaired cell proliferation and differentiation, and disturbances in various embryonic signaling pathways.[5,7,8] However, most previous studies and reviews have discussed these mechanisms separately, focusing only on specific pathways. In reality, these pathways are likely interconnected and may contribute collectively to abnormalities in neural tube development. Therefore, an understanding of the integrated mechanisms involved in VPA-induced NTDs remains limited and requires a more comprehensive evaluation to clarify the interactions among the pathways underlying the pathogenesis of this condition.

Due to the limited number of studies investigating the mechanism of HDAC inhibition in valproic acid exposure, this review does not discuss this mechanism in detail. Based on this limitation, this review focuses on examining and integrating the various molecular mechanisms involved in valproic acid-induced neural tube defects. In addition, this review seeks to elucidate the relationships and interactions among the mechanistic pathways that may contribute to neurulation failure and the development of neural tube defects. By integrating these mechanisms, this review is expected to enhance current understanding, provide a more comprehensive understanding of valproic acid-induced neural tube defects, and serve as a foundation for future research development and preventive strategies.

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Method

Protocol and Registration

This systematic review was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020. The study protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD420261384605).

Eligibility Criteria

The literature search strategy was developed using the PICO framework (Population, Intervention, Comparison, and Outcome).

Sources and Search Strategy

The literature search was done using PubMed, ScienceDirect, Wiley, and ProQuest as databases using the keywords molecular pathogenesis, valproate, neural tube defects, apoptosis, gene expression, and folate pathway. Five authors conducted the search strategy based on the availability of each database. The retrieved studies were then selected based on the title, abstract, and full text in accordance with the eligibility criteria that had been set.

Selection Process

All records that have been found will be imported into a reference manager, and duplicates will be removed.

Table 1. PICO Framework

Population	Intervention	Comparison	Outcome
All experimental embryonic models (animal-based or cell-based), including mammalian embryos or neural stem/progenitor cell lines, exposed to valproic acid and used to model neural tube defects or related neurodevelopmental disruption (all species, all sexes).	Experimental exposure to valproic acid in embryonic models, including in vivo and in vitro systems. All dosages, timing (e.g., pre-, peri-, or post-neurulation), durations, and frequencies of exposure are eligible, provided the study evaluates molecular, epigenetic, or developmental outcomes relevant to neural tube defects.	Non-exposed controls (no valproic acid exposure) and/or exposure to alternative anti-epileptic drugs, where used as a comparator to assess molecular, epigenetic, or developmental outcomes related to neural tube defects.	Eligible outcomes include changes in apoptosis-related markers, folate-pathway alterations, oxidative stress, epigenetic changes, dysregulation of developmental signaling pathways or gene expression (e.g., Wnt, Pax3, retinoic acid signaling), and morphological or phenotypic evidence of the neural tube defects or impaired neurulation.

Study selection will be conducted independently by the review team according to the predefined eligibility criteria in two sequential phases: (1) title and abstract screening; and (2) full-text screening. Five reviewers will participate in the screening process, with each record being assessed by at least two reviewers. Discrepancies will be discussed until the conflicts have been resolved; a third reviewer will decide if an agreement cannot be achieved. During screening, exclusion criteria will be applied in the following order:

- Not an experimental embryonic model (animal or cell-based)
- No exposure to valproic acid
- No relevant outcomes related to neural tube defects or molecular/epigenetic mechanisms
- No comparator (if required for analysis)
- Non-original research (reviews, editorials, conference abstracts without sufficient data)

Data Collection Process

Data extraction will be performed independently by at least two of five reviewers using a standardized extraction form. Discrepancies will be discussed with a third reviewer until resolved. Data will be extracted from text, tables, and figures; where necessary, digital tools will be used to extract numerical data from graphs. Authors may be contacted for missing or unclear data when required. Data extraction will include study type (in vivo/in vitro), experimental design (controlled), number of groups, sample size, presence and type of comparator, and duration of study; details of valproic acid exposure, including dose/concentration, timing (e.g., gestational stage or differentiation phase), duration, frequency, and route

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of administration; molecular and gene expression changes relevant to neurulation (e.g., Wnt signaling, Pax3, folate-pathway genes). Data will be extracted as continuous variables (e.g., mean ± SD, fold change, relative expression levels) or equivalent reported metrics. Where necessary, values will be standardized or normalized across studies. Outcomes also include morphological or phenotypic measures of neural tube defects, such as incidence of neural tube defects (e.g., spina bifida, anencephaly), reported as dichotomous data (e.g., number of affected embryos/total). Additional outcomes may include markers of oxidative stress, apoptosis, or related developmental pathways.

Table 2. Search Strategy

Database	Search Strategy	Search Result
PubMed	("Valproic Acid"[MeSH] OR "Valproate") AND ("Histone Deacetylase Inhibitors"[MeSH] OR "HDAC inhibition") AND ("Neural Tube Defects"[MeSH] OR "Spina Bifida" OR "Anencephaly") AND ("Molecular Biology" OR "Gene Expression Regulation" OR "Epigenomics")	2
Science Direct	Valproate AND "HDAC inhibition" AND "Neural Tube Defects" AND "Molecular Mechanism" AND "Epigenetics"	14
Wiley	(Valproate OR "Valproic Acid") AND (Epigenetic* OR "Histone Acetylation" OR "Gene Expression") AND ("Neural Tube Defects" OR "Congenital Malformations" OR "Neurodevelopment")	1350
ProQuest	noft((Valproate OR "Valproic Acid")) AND noft((Epigenetic* OR "Histone Acetylation" OR "Gene Expression")) AND noft(("Neural Tube Defects" OR "Congenital Malformations" OR "Neurodevelopment"))	7

Result

Study Selection

A total of 1373 records were identified through database searching. After removing duplicates and records marked as ineligible by automation tools, 30 studies remained for title and abstract screening. A total of 23 records were excluded due to the ineligible population, intervention, comparison, and outcomes. Ultimately, 7 studies were included in this systematic review.

Study Characteristics

The seven included studies, published between 1996 and 2025, investigated the molecular mechanisms of valproic acid (VPA) induced neural tube defects (NTDs) using both in vivo and in vitro experimental models. Most studies used in vivo mouse models, particularly CD-1, SWV, and LM/Bc mice, while one study used a chicken embryo model. Two studies used in vitro stem cell models, including P19C5 stem cells and mouse embryonic stem cells. VPA exposure was generally administered during early embryonic development, the critical period for neural tube formation.

The studies mainly examined mechanisms related to apoptosis, folate metabolism, retinoic acid (RA) signaling, oxidative stress, HDAC inhibition, and developmental gene regulation. Several studies reported that VPA altered the expression of important developmental genes, including Casp3, Bax, Trp53, MTHFR, folr1, Hoxa1, and Pax3. In addition, some studies also evaluated interventions or modulators, including IFN-gamma, LE135, and N-acetylcysteine (NAC), which demonstrated potential in reducing the harmful effects of VPA exposure and lowering the incidence of neural tube defects. Involving IFN-gamma studies reported a reduction in VPA-induced NTD incidence, reflecting the

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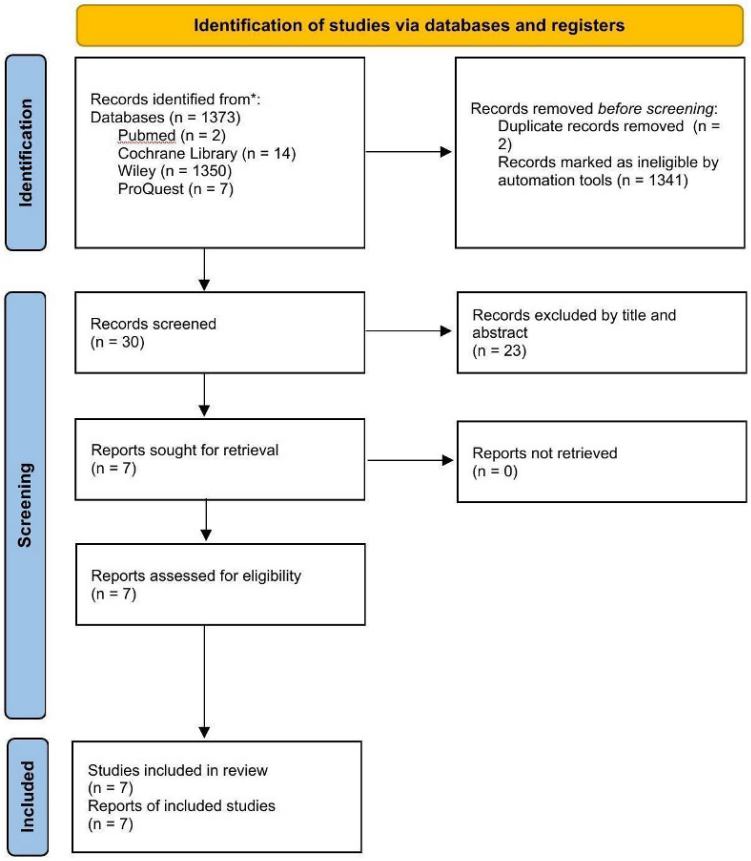

Figure 1: PRISMA Flowchart

effect of the intervention rather than VPA itself.

In addition, genetic background was found to influence the risk of VPA-induced NTDs. Studies comparing SWV and LM/Bc mice showed that the SWV strain had a higher risk of developing NTDs due to greater suppression of folate-related genes. Overall, the included studies showed that VPA disrupts multiple molecular pathways involved in neural tube development, whereas several studies evaluated interventions or modulators to reduce its teratogenic effects.

Risk of Bias Analysis

The included studies' methodological quality varies according to the SYRCLE and modified SYRCLE Risk of Bias scores.[16] Overall, the majority of studies revealed a low to moderate risk of bias in several areas, especially when it came to data completeness and outcome reporting. However, due to inadequate methodological reporting, several domains—especially those about randomization processes, allocation concealment, blinding, and variations from intended interventions—were often rated as unclear.

Many studies showed more consistent methodological quality and a comparatively decreased risk of bias, especially in areas of outcomes analysis and selective reporting. However, there was still uncertainty in areas like sequence generation, random housing, and blinding investigators or result assessors. On the contrary, multiple studies revealed greater issues across several categories, especially about experimental allocation procedures, insufficient outcome data, and a lack of transparency in the reporting of experimental methodologies.

Compared to more recent publications, older studies usually showed a higher likelihood of bias, primarily as a result of lax reporting guidelines and insufficient methodological procedure descriptions. Several "unclear" judgments in both SYRCLE and modified SYRCLE assessments were caused by common constraints found in all of the included studies, including inadequate information on allocation techniques, housing circumstances, and blinding protocols. Overall, most studies were deemed to have adequate evidence quality despite these methodological constraints; however, methodological rigor and reporting transparency still need to be improved.

Table 3. Characteristic Table

Author (Year)	Study Model	VPA Dosage & Timing	Main Molecular Target	Key Gene Alterations	Intervention/ Modulator	Main Phenotypic Outcome
Frascella et al. (2025)[9]	In vivo (CD-1 Mice)	400 mg/kg at GD 9:0	Apoptosis & Maternal Immune Stimulation (MIS)	Downregulation of Casp3, Bax, Bak1, Trp53 (after MIS)	Interferon-gamma (IFN-gamma)	Reduced NTD incidence & suppressed apoptotic genes.
Li et al. (2016)[10]	In vitro (P19C5 Stem Cells)	0.5–1.0 mM (Therapeutic range)	Retinoic Acid (RA) Signaling	Overexpression of Cdx1, Hoxa1, Cyp26a1	RA receptor antagonist (LE135)	Inhibition of EB axial elongation & patterning.
Mallela & Hrubec (2012)[11]	In vivo (CD-1 Mice)	400 mg/kg at GD 9:0	Apoptosis	Enhanced apoptotic cell population along the neural tube (TUNEL+)	IFN-gamma (Nonspecific MIS)	54% reduction in VPA-induced NTDs.
Finnell et al. (1997)[12]	In vivo (SWV vs LM/ Bc Mice)	400 mg/kg at GD 8:18	Folate Pathway & Genetic Susceptibility	Suppressed FBP-1 in sensitive strain; reduced MTHFR expression levels	Strain-dependent (Genetic background)	Higher NTD rate in SWV strain due to folate gene suppression.
Jergil et al. (2011)[13]	In vitro (Mouse Embryonic Stem Cells)	0.5 mM (Short-time exposure)	Toxicogenomic Response	Alteration in Wnt, Notch, and Hox gene clusters	VPA Analogs (Valpromide, etc.)	Prediction of developmental neurotoxicity via gene profiles.
Wlodarczyk et al. (1996)[14]	In vivo (SWV vs LM/ Bc Mice)	400 mg/kg at GD 9:0	Transcription Factors & Cell Cycle	Elevation of c-fos, c-jun, CREB and overexpression of p53 and wee-1	N/A	Accelerated developmental gene profile in sensitive strains.
Hsieh et al (2013)[15]	In vivo (chicken embryo model)	30 mM, 100 µL/egg at HH stage 22 (day 3.5)	Folate pathway, HDAC inhibition & oxidative stress	Downregulation of folr1, IGF2R, RGS2, COL6A3, EDNRB, KLF6, and Pax3	VPA as intervention, N-acetylcysteine (NAC) as modulator	Dysregulated neurulation-related gene expression associated with increased neural tube defect susceptibility.

Table 4. Risk of bias for in vivo studies (SYRCLE RoB tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Frascella et al. (2025) [9]	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Finnell et al. (1997) [12]	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Mallela & Hrubec (2012) [11]	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Wlodarczyk et al. (1996 [14]	Unclear	Yes	Unclear	Unclear	Unclear	No	Unclear	Yes	Unclear	Unclear
Hsieh et al. (2013) [15]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes

Table 4. Risk of bias for in vitro studies (Modified SYRCLE's RoB tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Jergil et al., (2011) [13]	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Unclear
Li et al., (2016) [10]	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes

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Discussion

This systematic review utilized seven studies, consisting of five in vivo studies and two in vitro studies. The majority of in vivo studies used a VPA dose of 400 mg/kg at GD 8.18-9.0, while in vitro studies used concentrations of 0.5–1.0 mM.

Alteration of Apoptotic Pathways and Cellular Balance

Two in vivo studies showed that exposure to VPA increased apoptosis along the neural tube. Frascella discovered that 400 mg/kg VPA treatment at gestational day 9.0 decreased apoptosis-related genes such as Casp3, Bax, Bak1, and Trp53 after maternal immunological activation with IFN-γ. This intervention was found to reduce the incidence of NTDs. Similarly, Mallela reported more TUNEL-positive cells during VPA treatment, indicating increased apoptotic cell populations along the neural tube. IFN-γ activated the mother's immune system, causing a 54% reduction in VPA-induced NTDs.

Disruption of Developmental Transcription and Folate Transport

Through an in vivo mouse model study, Wlodarczyk et al. (1996) found that VPA exposure increased the expression of transcription factor genes such as Pax-3, Emx-1, Emx-2, c-fos, c-jun, and CREB in SWV embryos. Wlodarczyk explained that Pax3 is expressed in regions of the neural tube where neural crest cells emerge and migrate, whereas c-jun and c-fos are involved in cell proliferation. In addition, bcl-2 mediates the growth-inhibitory and apoptotic effects associated with p53, while p53 plays an important role in regulating neural tube closure through cell cycle regulation. In LM/Bc embryos, VPA exposure increased the expression of bcl-2 and p53 while decreasing Pax-3 expression during neural tube closure. Furthermore, the expression ratio of bcl-2 to p53 was found to be higher for bcl-2, suggesting that VPA primarily inhibits neuroepithelial cell proliferation. These findings are important because they suggest a shift in the understanding of teratogenic mechanisms, from the assumption that teratogens induce cell death to the concept that teratogens such as VPA disrupt the balance between proliferation and differentiation. Furthermore, Hsieh et al. (2013) expanded these findings by identifying 21 genes disrupted by VPA, including five major interacting genes potentially associated with neural tube defects (NTDs), namely IGF2R, RGS4, COL6A3, EDNRB, and KLF6. Gene ontology analysis showed that the affected genes are involved in translation, signal transduction, transcription, cell adhesion, neural cell migration, transport, and organismal development. Additionally, expression of the folate receptor gene (folr1) by VPA was reported, suggesting a molecular link between VPA exposure and folate deficiency. Taken together, the findings of Wlodarczyk et al. and Hsieh et al. suggest that VPA disrupts neurulation through alterations in developmental transcription factors and folate-related pathways.[17] These molecular disturbances impair essential cellular processes required for neural tube formation. In addition to these mechanisms, accumulating evidence suggests that disruption of retinoic acid (RA) signaling represents another important pathway contributing to VPA-induced teratogenicity.

Retinoic Acid Signaling and Developmental Gene Dysregulation

In an in vitro study using P19C5 stem cells, Li reported that therapeutic VPA doses (0.5-1.0 mM) altered retinoic acid (RA) signaling pathways, which resulted in upregulation of developmental genes such as Cdx1, Hoxa1, and Cyp26a1. Furthermore, treatment with the RA receptor antagonist LE135 reduced embryoid body axial elongation and patterning, indicating that abnormal RA signaling contributes to poor embryonic development. Using a P19C5 embryoid body (EB) model, Li stated how EBs that were not exposed to VPA exhibited normal axial elongation, whereas treatment with 0.6 mM VPA inhibited elongation. RNA levels of RA target genes and the trend of increased Cdx1 expression from 0.5 to 1.8, and Hoxa1 expression from 0.4 to 1.4, on the first day of treatment were spotted. Cdx1, Hoxa1, and Cyp26a1 are RA target genes that collectively function to maintain retinoic acid signaling homeostasis during gastrulation and neurulation. However, when assessed using the RARE-Luc reporter assay, RA levels did not increase, indicating that VPA does not elevate RA concentrations. Therefore, the increased expression of Hoxa1 and Cdx1 was not caused by elevated RA levels, but rather by histone hyperacetylation, which renders chromatin more responsive to RA signaling. Consistent with these findings, VPA exposure has been shown to functionally mimic retinoic acid excess by aberrantly activating RA signaling pathways.[18] This dysregulation promotes the overexpression of key caudalizing genes, including

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Cdx1 and Hoxa1, which are essential for determining positional identity along the embryonic axis.[19]

Mechanistically, Jergil et al. (2011) showed that VPA induces rapid toxicogenomic shifts in Hox, Wnt, and Notch gene clusters within hours of exposure. This suggests that VPA interferes with the "epigenetic clock" of the embryo. By causing a premature or exaggerated expression of these genes, VPA disrupts the precisely timed coordination of cell migration and differentiation required for neural fold elevation. This "molecular chaos" prevents the neural folds from meeting at the midline, leading to the physical manifestation of neural tube defects (NTDs) and associated axial skeletal malformations.[20] Taken together, the findings of Li et al. and Jergil et al. suggest that VPA disrupts embryonic development through coordinated alterations in retinoic acid (RA) signaling and epigenetic regulation. These molecular disturbances impair the tightly regulated processes of neural patterning, cell differentiation, and neural tube closure, thereby contributing to the development of neural tube defects (NTDs). Although previous studies have demonstrated that VPA disrupts multiple molecular pathways involved in neurulation, not all fetuses exposed to VPA develop neural tube defects (NTDs). This observation suggests that the embryonic response to VPA exposure is influenced not only by the molecular effects of VPA itself but also by interindividual differences in genetic susceptibility.

Genetic Susceptibility: Determinants of Embryonic Resilience

Finnell et al. (1997) demonstrated this by comparing two mouse strains administered the same dose of VPA and found that the SWV strain was highly susceptible to NTDs, whereas the LM/Bc strain was more resistant. In the susceptible SWV strain, VPA reduced the expression of the FBP-1 (folate-binding protein-1) gene and did not alter MTHFR expression, resulting in folate deficiency. In contrast, in the resistant LM/Bc strain, VPA increased the expression of both FBP-1 and MTHFR genes, which may protect against the teratogenic effects of VPA. Findings from Finnell suggest that individuals who can upregulate folate pathway gene expression in response to VPA exposure may be protected from NTDs. Similarly, Wlodarczyk et al. (1996) reported that in the susceptible SWV strain, VPA decreased the expression of the bcl-2 and p53 genes, leaving neuroepithelial cells without adequate protection against the teratogenic effects of VPA. In contrast, in the resistant LM/Bc strain, VPA increased the expression of bcl-2 and p53. These findings indicate that individuals capable of increasing bcl-2 expression in response to the teratogenic effects of VPA may exhibit greater resistance to NTDs. Taken together, the findings of Finnell et al. and Wlodarczyk et al. suggest that the teratogenic effects of VPA are influenced not only by specific molecular pathways but also by the embryo's genetic capacity to activate compensatory responses. Therefore, genetic susceptibility appears to be a key determinant of embryonic resilience against VPA-induced neural tube defects (NTDs). This genetic resilience aligns with the insights from Mallela and Hrubec (2012) regarding the reduction of VPA-induced NTDs. Rather than suggesting that VPA itself decreases the defect risk, their study clarifies that the reduction is strictly driven by maternal immune stimulation, which effectively mitigates VPA-induced teratogenicity by normalizing dysregulated cellular apoptosis and preventing malformations. A more comprehensive analysis of this maternal immune modulation is provided in the following section.

The One-Carbon Metabolism: MTHFR and FBP-1 qs Protective Buffer

The genes MTHFR (5,10-methylenetetrahydrofolate reductase) and FBP-1 (Folate Binding Protein-1) are central to this protective mechanism. In the highly sensitive SWV strain, VPA insult caused a significant suppression of MTHFR and FBP-1 mRNA expression, effectively "strangling" the embryo's ability to process folate.[21] This metabolic failure leads to localized folate deficiency at the neural folds, even if maternal folate levels are normal. In contrast, resistant strains maintain stable or up-regulated expression of these genes, allowing them to maintain the DNA synthesis and methylation reactions necessary for neural tube closure. This underscores that VPA teratogenicity is not a "one-size-fits-all" phenomenon but is dictated by a complex interaction between the drug and the host's metabolic genetic blueprint malformations.[17]

One of the most novel findings of this review comes from the study by Frascella et al. (2025), which explored the role of maternal immune stimulation (MIS) in modulating teratogenic effects caused by VPA. This study suggests that modulation of MIS may partially protect embryos from valproate-induced NTDs. This study showed suppressed apoptotic genes through the downregulation of Casp3, Bax, Bak1, Trp53, and lower NTD incidence after stimulation with interferon-gamma (IFN-γ), with the VPA group having 45.3%

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incidence of NTDs, and the IFN-γ group at 21.2%. These significant changes suggest that MIS may influence embryonic resilience against VPA-induced teratogenicity, particularly through the apoptotic pathway. Mechanistically, MIS may modulate these effects by altering the inflammatory signaling, oxidative stress responses, or modulating downstream apoptotic cascades. However, these findings indicate that a reduction in NTD incidence was observed only when maternal immune stimulation (MIS) with interferon-gamma (IFN-γ) was administered concomitantly with VPA exposure. Therefore, the observed protective effect is more likely attributable to the ability of MIS to modulate apoptotic pathways. In contrast, VPA itself remains a well-established teratogen associated with an increased risk of neural tube defects (NTDs). This combined perspective demonstrates that the pathological cascade driven by VPA-induced folate disruption and oxidative stress ultimately converges into widespread embryonic apoptosis, a mechanism that is directly countered by the protective pathways activated during maternal immune stimulation.[23]

Clinical Implications

Although current clinical guidelines recommend avoiding valproate during pregnancy because of its well-established teratogenicity, its clinical use is strictly contraindicated during pregnancy due to its well-established, severe teratogenic risks. Avoiding valproate use for childbearing patients is heavily mandated, especially whenever effective alternatives such as lamotrigine or levetiracetam are available to minimize the risk of fetal malformations while maintaining maternal seizure control. Consequently, a small subset of women whose seizures cannot be adequately controlled with alternative antiseizure medications may still require continued valproate therapy after careful individualized risk-benefit assessment.[22] This presents a major clinical dilemma for women of childbearing age, where discontinuing valproate may endanger both the mother and the fetus through uncontrollable seizures, while continuation increases teratogenic risks.[5] The present review suggests that VPA-induced neural tube defects arise through interconnected mechanisms involving apoptosis, folate-pathway disruption, oxidative stress, retinoic acid signaling, and dysregulation of developmental gene expression. Therefore, MIS-related findings may open future opportunities for preventive therapeutic strategies by demonstrating that maternal immune stimulation may attenuate apoptotic gene expression and reduce neural tube defect incidence in experimental models. Although this strategy is still highly experimental and cannot be applied clinically at present, modulation of the maternal immune pathway can be considered as an adjunctive protective approach for pregnant patients with generalized epilepsy. Further mechanistic studies are necessary before clinical application can be considered.

Strengths and Limitations

This review integrates multiple domains involved in valproate-induced NTD pathogenesis, including apoptosis, folate-pathway dysregulation, oxidative stress, epigenetic alteration, and developmental signaling abnormalities. This provides broad molecular synthesis across diverse experimental systems. The inclusion of both in vivo and in vitro embryonic models provides a comparison of molecular responses across species. This review also highlights potential therapeutic strategies for generalized epilepsy, such as maternal immune stimulation, which is currently still underexplored in current literature. However, limitations in this review should be acknowledged. Heterogeneity was still present among included studies regarding animal species, developmental stage, valproate dosage, timing of exposure, and outcome measurements. These limitations restricted direct comparisons between studies.

Further Directions

The findings of this review reinforce that valproic acid-associated teratogenicity is multifactorial, involving effects such as apoptosis, folate dysregulation, oxidative stress, and altered developmental gene signaling. An understanding of these effects may contribute to more thorough planning of preventive strategies in pregnant patients requiring antiseizure medication. Caution regarding valproate use during pregnancy is still needed, particularly during early neurulation. However, because some patients with generalized epilepsy cannot achieve adequate seizure control with alternative antiseizure medications, valproate may remain necessary in selected cases despite its teratogenic risk. Therefore, identifying protective interventions is needed. Future studies should focus on therapeutic strategies capable of reducing teratogenic risk while still controlling seizures effectively. In particular, the emerging evidence surrounding MIS shows potential for further investigation as a possible therapeutic target. Standardized exposure controls

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and approaches also need to be considered to improve mechanistic understanding and to support the development of safer therapeutic strategies for pregnant women who require valproate.

Conclusion

This systematic review shows that neural tube defects (NTDs) triggered by valproic acid (VPA) are mediated by several interrelated mechanisms, including VPA-induced oxidative stress, apoptosis, folate-pathway dysregulation, epigenetic modification, and disruption of developmental signaling pathways such as retinoic acid signaling. Studies conducted in vitro and in vivo consistently show that VPA modifies the expression of genes necessary for cellular homeostasis, neurulation, and embryonic patterning.

The results also imply that teratogenic outcomes are influenced by genetic vulnerability, specifically through variations in folate metabolism and anti-apoptotic gene responses. Although this method is still experimental, recent studies on maternal immune stimulation (MIS) suggest a possible protective function against VPA-induced teratogenicity.

Overall, it seems that host susceptibility factors and molecular signaling disturbance interact intricately to cause VPA teratogenicity. To support the development of safer therapeutic and preventive approaches for pregnant patients in need of VPA treatment, more mechanistic and standardized experimental research is required.

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The authors declare that no declarations are relevant to this systematic review.

Author's Contribution

All authors contributed equally to this review. The tasks were fairly distributed, including literature screening, data analysis, and manuscript drafting. Each author was responsible for specific parts of the writing and revision process. All authors read and approved the final manuscript.

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