REVIEW ARTICLE

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Mechanism of Valproic acid Induced Autism: Canonical Wnt-β-Catenin Pathway

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Abstract

Valproic acid (VPA) is anti-epileptic and mood stabilizer drug that induces autism spectrum disease (ASD). However, VPA has several side effects; hepatic steatosis, hepatotoxicity, hemorrhagic pancreatitis, encephalopathy, bone marrow suppression and metabolic disorders such as obesity. VPA proved to be unavoidable and could not be excluded in epileptic pregnant women. Non-controlled epileptic attacks during pregnancy produce high risk of injury to both mother and fetus. However, VPA crosses the placenta and accumulate in the fetal circulation with higher concentration than that in the maternal blood, causing toxicity and teratogenicity. Gestational VPA treatment for a life-threatening epilepsy caused numerous defects in children, including neural tube defects, intellectual impairments and cognitive-behavioral impairments. 8.9% of children exposed to VPA in utero develop autistic features. VPA exposure in the first trimester of gestation represented the highest risk for the child to develop autism, showed classical signs of autism, and developmental and behavioral delays. The full mechanisms of VPA are not fully elicited. This review discusses canonical Wnt/β-Catenin pathways as possible mechanism involved in autism induction upon VPA use.

Keywords: Valproic acid; Autism; Canonical Wnt/β-Catenin pathway.

1. Introduction

1.1. Background

Autism spectrum disease (ASD) is a complex, functional, multifactorial and one of the fastest growing neurodevelopmental and pervasive disorder. ASD is a childhood onset and mainly diagnosed within the first three years of life **(Benger et al., 2018)**. It represented a significant public health problem and a huge burden for education and social service systems **(Järbrink and Knapp, 2001)**. The Autism and Developmental Disabilities Monitoring Network estimated ASD prevalence at 1 in 54 children aged 8 years **(Maenner et al., 2020)**.

Abnormal development is often observed in autistic patients in the early stages of life; weight fluctuation, abnormal brain development **(Chen et al., 2015)**, disruption in synaptic connection and hyperactive neuronal connections resulting in behavioral complexities **(Speed et al., 2015)**. ASD pathology involves early accelerated brain growth

with large brain size and either hyper-reactivity indicated by aggressive behaviors or it involves hypo-functionality of the brain and inhibitory and negative behaviors **(Shafique, 2018)**.

The pathophysiology of ASD is poorly understood, but evidence indicated that a strong genetic component and the environment act in concert as triggering factors **(Ebihara et al., 2017)**.

Valproic acid (VPA) is generally regarded as a first-choice agent for most forms of idiopathic and symptomatic generalized epilepsies; tonic-clonic, myoclonic and absence seizures **(Perucca, 2002)**. VPA also used for migraine headaches prophylaxis, mood stabilizer **(Nicolini and Fahnestock, 2018)**, and to treat manic episodes related to bipolar disorder in humans **(Roullet et al., 2013)**.

VPA proved to be unavoidable and could not be excluded in epileptic pregnant women **(Stephen et al., 2012)**. Non-controlled epileptic attacks during pregnancy produce high risk of injury to both mother and fetus **(Klein, 2011)**. However, embryonic exposure of VPA associated with congenital malformations **(Ornoy, 2009)**, cognitive impairments as well as ASD **(Smith and Brown, 2014)**.

2. Pathophysiological mechanisms of autism induced by VPA

Despite, full mechanism by which exposure to VPA causes autistic-like behaviors in both humans and rodents is not entirely known. Multiple pathophysiological events were proposed to define the autistic effect of VPA.

2.1. The contribution of acetylcholine (ACh)

Neurotransmitters control some of the behavioral impairments distinctive to ASD **(Guo and Commons, 2017)**. ACh is synthesized with acetyl CoA and choline by the action of choline acetyl transferase (ChAT) **(Kroner, 2009)**. ACh is hydrolyzed by acetylcholinesterase (AChE) enzyme at the central and peripheral cholinergic synapses into its two component parts, acetic acid and choline **(Block and Beale, 2004) (Fig. 1)**.

The dysregulation of these enzymes and cholinergic receptors (both muscarinic and nicotinic acetylcholine receptors) caused many neurological disorders; alzheimer's disease (AD), Parkinson's disease, schizophrenia, and ASD **(Sarter et al., 2012)**.

Dysregulation of cholinergic system commonly observed in the brain of ASD patient. Genetic mutations in CHRNA7 (encoding a7-nicotinic acetylcholine receptor subunit) and CHRM3 (encoding M3 muscarinic receptor) were found in autistic patients **(Petersen et al., 2013)**. In post-mortem studies with ASD patients' brain, M1 muscarinic receptor and several nicotinic receptors subunits (a3, a4, b2) were reduced **(Ray et al., 2005)**. Decreased choline peak was observed in the gray matter and temporal lobe of ASD patients **(Friedman et al., 2006)**. Prenatal VPA exposure enhanced acetylcholinesterase protein levels **(Kim et al., 2014)**.

2.2. Canonical Wnt/β-Catenin Pathways

Genetic factors play a major role in the risk for neurodevelopmental disorders; ASDs **(Kwan et al., 2016)** which had heritability estimates greater than 90% suggesting a strong genetic component to disease **(Lichtenstein et al., 2010)**.

Wingless and integration site (called Wnt) pathway is a cascade of several signaling implicated in development, growth, metabolism and maintain of stem cells **(van Amerongen and Nusse, 2009)**. The canonical Wnt pathway regulates multiple physiological and pathological processes including angiogenesis, inflammation, and fibrosis. As well as playing a variety of roles in almost all tissues and a central role in the development and regulation of the CNS and several genes belonging to the cascade genetically associated with ASDs **(Caracci et al., 2016)**. The Wnt/β-catenin signaling plays an important role in cell fate, driving cell proliferation, epithelial-mesenchymal transition (EMT) signaling, and embryonic development **(Bienz and Clevers, 2000)**.

Canonical Wnt ligands are secreted by neurons and immune cells in the CNS (**Marchetti and Pluchino, 2013)**. Dysregulation of the core neurodevelopmental pathways is associated with the clinical presentation of ASD, and one of the major pathways involved in developmental cognitive disorders is the canonical Wnt/β-catenin pathway **(Kwan et al., 2016)**. Several genetic mutations observed in ASD are linked with the deregulation of the canonical Wnt/β-catenin pathway **(Krumm et al., 2014)**. Canonical Wnt/β-

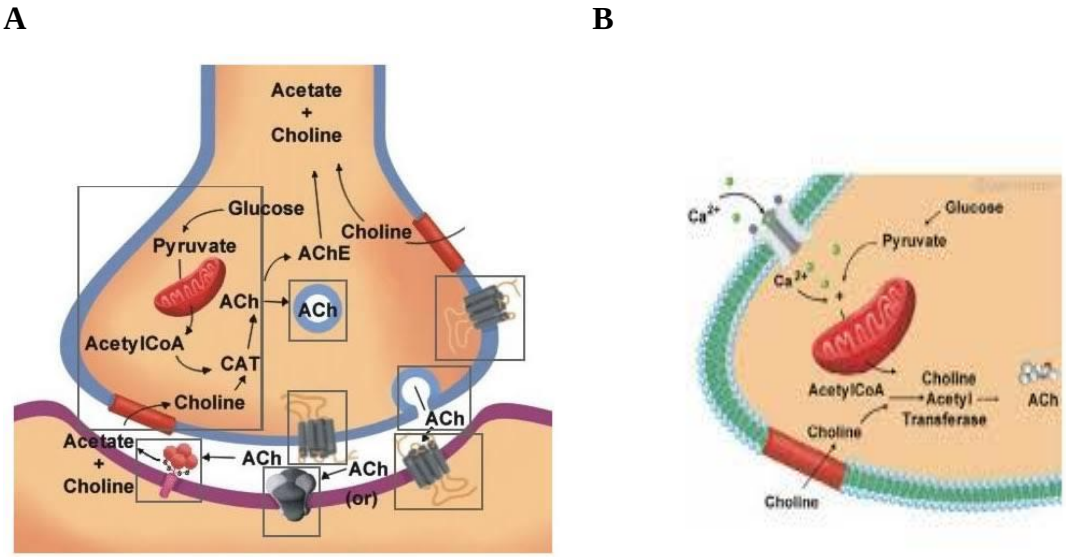

Figure 1: Synthesis and degradation of ACh. ACH: Acetylcholine; CAT: Choline acetyltransferase.

catenin pathway, has a critical role in the development of the CNS, involved in and upregulated in ASD **(Mulligan and Cheyette, 2017)**.

2.2.1. Transcription factor β-catenin/T-cell factor/lymphoid enhancer factor (TCF/LEF)

The major effector of the canonical Wnt pathway is the transcription factor β-catenin/T-cell factor/lymphoid enhancer factor (TCF/LEF). In the absence of Wnt, the free cytosolic β-catenin is phosphorylated and is tightly controlled by a destruction complex, consisting of AXIN, tumor suppressor adenomatous polyposis coli (APC), and glycogen synthase kinase 3b (GSK-3β). The destruction complex interacts with β-catenin and phosphorylates it **(Fig. 2A) (Schinner et al., 2008)**. The phosphorylated β-catenin is then degraded in the proteasome (β-catenin proteasomal degradation: CPD) **(Fig. 2A) (Pate et al., 2014)**. Activated GSK-3β downregulates β-catenin accumulation and its nuclear translocation **(Clevers and Nusse, 2012)**.

In the presence of Wnt ligands, the Wnt receptor interacts with the Frizzled (Fzd) receptor and LDL receptor-related protein 5/6 (LRP5/6) coreceptors **(Fig. 2B)**. The Wnt receptor associates with Dishevelled protein (Dsh). This triggers the disruption of the destruction complex and prevents CPD **(Welters and Kulkarni, 2008)**. β-catenin then translocates to the nucleus and interacts with TCF/LEF which stimulates the β-catenin downstream target genes (PDK, MTC-1, cMyc, cyclin D1, Cox 2, AXIN 2) **(Fig. 2B)** **(Lecarpentier et al., 2017)**. The canonical Wnt pathway controlled the expression of Wnt target genes by stabilizing cytoplasmic β -catenin **(Fig. 2B) (Nusse and Clevers, 2017)**.

2.2.2. Phosphatidylinositol 3 kinase/serine/threonine kinase (protein kinase B)/mammalian/mechanistic target of rapamycin (PI3K/Akt) pathway

Phosphatidylinositol 3 kinase/serine/threonine kinase (protein kinase B)/mammalian/mechanistic target of rapamycin (PI3K/Akt/mTOR) pathway is implicated in proliferation, growth, protein synthesis and metabolism **(Fig. 3) (Yu and Cui, 2016)**. Wnt/ β-catenin pathway, through the inhibition GSK-3β activity, is considered as one of the upstream main activators of PI3K/ Akt/mTOR pathway **(Chen et al., 2014)**. GSK-3β, a major inhibitor of the Wnt ligands **(Zhou et al., 2012)**, regulates numerous pathophysiological pathways **(Ambacher et al., 2012)**. In addition, the decrease of β- catenin levels down-regulates the expression of PI3K/ Akt/mTOR pathway **(Yue et al., 2010)**. Inhibition of GSK-3β, positively stimulates the canonical Wnt pathway **(Zhang et al., 2003)**.

β-catenin activation induces the expression of PI3K/Akt signaling **(Yue et al., 2010) (Fig 3)**. Increase rate of glucose metabolism is associated with the over activation of PI3K/Akt pathway **(Reuter et al., 2010)**. Activation of PI3K/Akt pathway stimulates the hypoxia inducible factor-1-alpha (HIF-1α), which induces stimulation of glycolytic enzymes such as glucose transporter-1

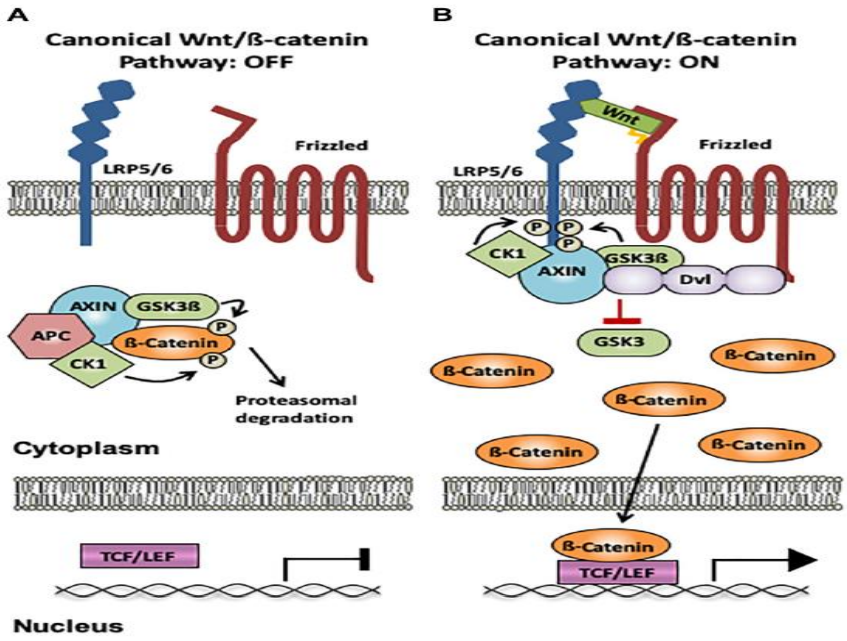

Figure 2: Wnt signaling pathways. (A) Canonical Wnt/β-catenin pathway OFF (B) Canonical WNT/ß-catenin pathway ON. CK1: casein kinase 1; GSK3β: glycogen synthase kinase 3b; Axin1: axis inhibition protein 1; APC: adenomatous polyposis coli; TCF: T-cell factor; LEF: lymphocyte enhancer-binding factor; LRP5/6: LDL receptor-related protein 5/6; Dvl: Dishevelled.

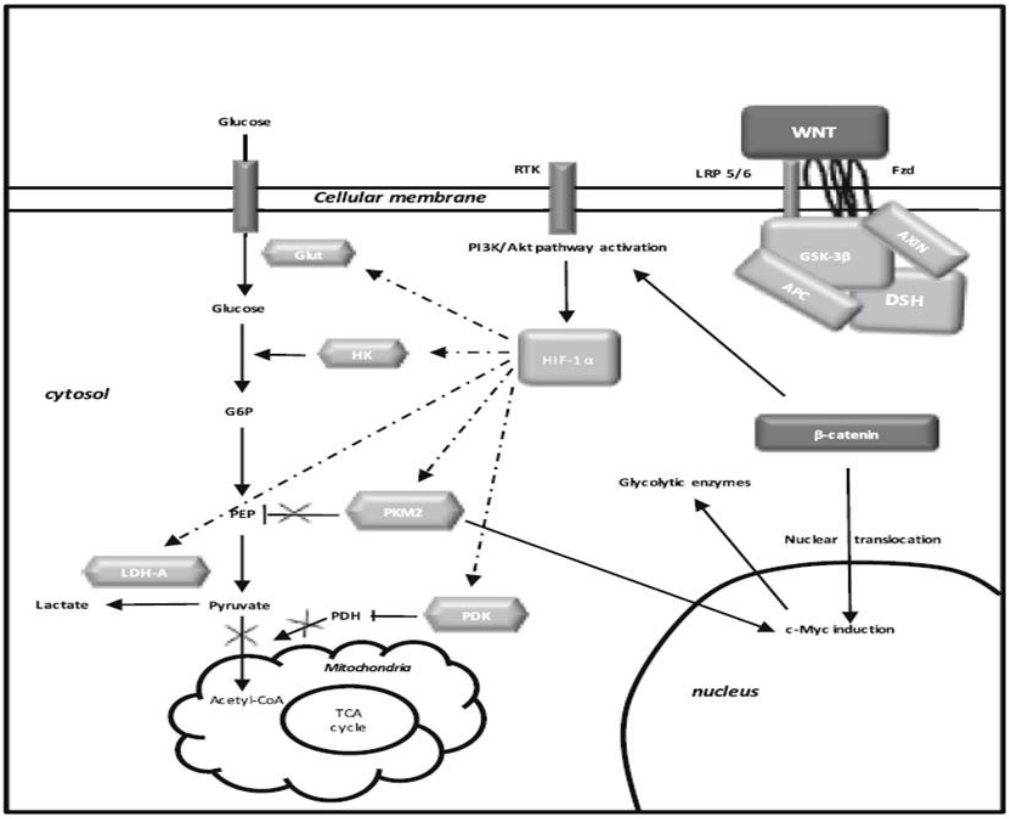

Figure 3: Relation between activated Wnt/β-catenin pathway and Warburg effect in ASD. GSK-3β: glycogen synthase kinase 3b; Axin1: axis inhibition protein 1; APC: adenomatous polyposis coli; LRP5/6: LDL receptor-related protein 5/6; GLUT: Glucose Transporter; LDHA: Lactate Dehydrogenase A; PDK: Pyruvate dehydrogenase kinase; c-Myc: Cellular Myeloctomatosis; PKM2: Pyruvate Kinase M2; PI3K: Phosphatidyl Inositol 3-Kinases; PDH: Pyruvate Dehydrogenase Complex; TCA: Tricarboxylic acid; Fzd: Frizzled; HIF-1α: Hypoxia-inducible factor -1α; Dsh: Dishevelled; PEP: Phosphoenol pyruvate; G6P: Glucose 6 phosphate.

(GLUT-1), lactate dehydrogenase A (LDHA), pyruvate dehydrogenase kinase (PDK1) and Pyruvate Kinase M2 (PKM2) **(Fig. 3) (Sun et al., 2011)**.

2.2.3. Phosphate and Tensin Homolog (PTEN)

PTEN, a gene located on chromosome 10q23, is involved in a wide variety of cellular processes relevant to brain growth and circuit function **(Kwon et al., 2006)**. PTEN is associated with ASD. PTEN has multiple functions, is a negative regulator of the PI3K/Akt/mTOR pathway results in behavioral abnormalities in ASD **(Lugo et al., 2014)**.

Several genetic variants in PTEN are observed in ASD patients **(Butler et al., 2005)**. PTEN mutant mice display abnormal social interactions, hyperactivity, excessive responses to external stimuli **(Ogawa et al., 2007)**; they also develop macrocephaly and neuronal hypertrophy in the CNS, similar to human patients **(Kwon et al., 2006)**.

PTEN deficiency in vivo increased the net excitatory drive onto granule neurons, enlarges the neuronal size, and increases the density of dendritic spines **(Luikart et al., 2011)**. The genes, PTEN which are negatively regulated by mTOR kinase activity, are responsible for syndromic ASD pathogenesis **(Tang et al., 2014)**.

The knockout of the gene encoding PTEN protein, a cytoplasmic protein suppressor of Wnt/β-catenin pathway, identified as a high-risk ASD susceptibility gene **(Frazier et al., 2015)**. Knockout of PTEN in Purkinje cells impaired social relation, behavior and deficits in motor learning **(Lugo et al., 2014)**.

2.2.4. Aerobic Glycolysis (Warburg Effect)

The Wnt pathway induces aerobic glycolysis allowing glucose utilization for cell proliferation **(Pate et al., 2014)**. A large proportion of the glucose supply is fermented in lactate regardless of the availability of oxygen. This phenomenon is called aerobic glycolysis or Warburg effect **(Warburg, 1956)**. As a consequence, a large part of cytosolic pyruvate is not converted into acetyl-CoA which does not enter the TCA cycle. PDK1, a key regulator of glycolysis, phosphorylates the PDH complex which partially inhibits the conversion of pyruvate to acetyl-CoA into mitochondria **(Fig. 3&4) (Lecarpentier et al., 2017).**

Cytosolic pyruvate is converted into lactate through activation of LDHA. Moreover, up regulation of MCT-1 diverts pyruvate towards lactate secretion from the cell **(Fig. 4) (Pate et al., 2014)**. Both PDK1 and the lactate transporter MCT-1 are Wnt/β-catenin targets. Moreover, the Wnt pathway induces the transcription of genes involved in cell proliferation, cMyc (through glutaminolysis, nucleotide synthesis, and LDHA activation) and cyclin D1 (through G1) **(Fig. 4) (Niehrs and Acebron, 2012)**. The Wnt target gene, cMyc, drives aerobic glycolysis and glutaminolysis **(Dang, 2010)**. cMyc also induces LDHA activation (for conversion of cytosolic pyruvate into lactate) **(Wise et al., 2008)**. cMyc also increases (HIF-1α) mediated control of PDK1 **(Fig. 4) (Lecarpentier et al., 2017)**.

2.2.5. Glucose Transporter-1 (GLUT-1)

Glucose is a vital metabolic fuel for all mammalian cells and major energy fuel of the central nervous system. Under normal physiological conditions cells are entirely dependent on a continuous supply of glucose and many other blood-borne nutrients. This process is mainly limited at the cellular level by the presence of impermeable cell membranes and at the tissue level by a barrier between tissues and their blood supply. Its availability is restricted given the selective permeability of the blood brain barrier (BBB) and the relative lack of carbohydrate stores in the brain. Thus, glucose transport across the BBB and into neural cells is critical for cerebral physiologic function and energy metabolism **(Chen et al., 2015)**. In some cases, this barrier prevents passive diffusion of glucose and other nutrients into and out of tissue and cells. As an alternative, glucose is transferred across the cell membranes and tissue barriers by a specific saturable transport process involving members of two different classes of glucose transporters, sodium-independent glucose transporters (facilitated transport; GLUT) and sodium-dependent glucose transporters (secondary active transport; SGLT) **(Shah et al., 2012)**.

GLUT-1 involved in neurodevelopmental disability **(Srour et al., 2017)**. Expression of different isoforms of both the GLUT and SGLT families has been shown to be cell type specific **(Thorens and Mueckler, 2010)**. The GLUT-1 gene is a key regulator of glucose transport into and out of the brain across the BBB acting to maintain CNS

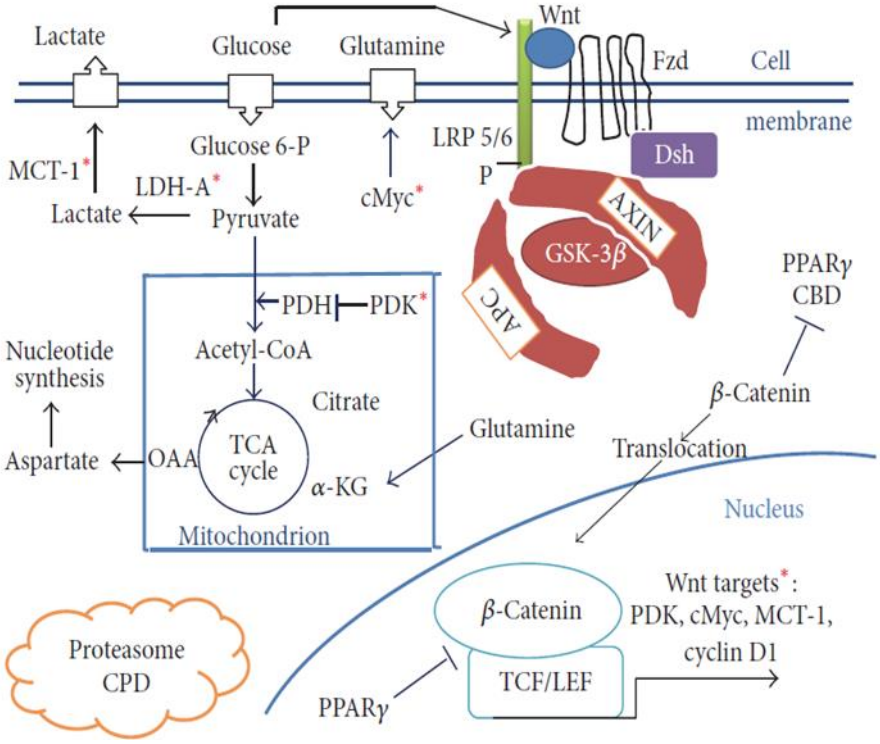

Figure 4: Interactions between the canonical Wnt/β-catenin pathway and PPAR-γ under aerobic glycolysis conditions. APC: adenomatous polyposis coli, CPD: β-catenin proteasomal degradation, CBD: catenin binding domain; Dsh: Dishevelled; Fzd: Frizzled; GSK-3β: glycogen synthase kinase-3beta; LDH: lactate dehydrogenase; LRP5/6: low-density lipoprotein receptor-related protein 5/6; MCT-1: monocarboxylate lactate transporter-1; PPAR-γ: peroxisome proliferator-activated receptor gamma; PDH: pyruvate dehydrogenase complex; PDK: pyruvate dehydrogenase kinase; TCF/LEF: T-cell factor/lymphoid enhancer factor; TCA: tricarboxylic acid ∗:Wnt targets: PDK, cMyc, MCT-1, and cyclin D1; GSK-3β: glycogen synthase kinase 3b; Axin1: axis inhibition protein 1; APC: adenomatous polyposis coli.

homeostasis **(Mann et al., 2003)**.

Many studies confirmed the presence of GLUT-1 protein in brain microvascular endothelial cells as well as in astrocytes **(Virgintino et al., 1997)**. GLUT-1 is the key regulator of glucose across the BBB **(Chen et al., 2015)**. GLUT-1 and GLUT-3 are mainly important for the insulin-sensitive homeostasis of glucose transport **(McEwen and Reagan, 2004)**. Then, the conversion of phosphoenolpyruvate (PEP) and ADP into pyruvate is the final step in glycolysis after glucose entered the cell **(Fig. 3)**. The enzyme pyruvate kinase (PK) catalyzes this reaction. PK have four isoforms: PKM1, PKM2, PKL, and PKR. The dimeric form of PKM2 has low affinity with PEP **(Christofk et al., 2008)**. Under high glucose concentration, PKM2 is translocated to the nucleus **(Harris et al., 2014)**, which reduces its activity and targets PKM2 toward lysosome-dependent degradation **(Lv et al., 2011)**. Nuclear PKM2 binds nuclear β-catenin and then induces c-Myc mediated expression of glycolytic enzymes including GLUT, LDHA, PDK1, and PKM2 **(Fig. 3& 4) (Yang et al., 2012)**. Activated c-Myc also activates glutaminolysis and tends to nucleotide synthesis **(Wise et al., 2008)** by activating HIF-1α which controls PDK1 **(Fig. 4) (Kim et al., 2007)**.

2.2.6. Interactions between Peroxisome Proliferator-Activated Receptor-Gamma (PPAR-γ) and canonical Wnt/β-catenin pathway

PPARs alpha, beta/delta, and gamma (α, β, δ & γ) are ligand-activated transcriptional factors which belong to the nuclear hormone receptor superfamily. with distinct physiological functions in regulating lipid and glucose metabolism, as well as inflammatory response and tissue distribution **(Barone et al., 2019)**. PPARs regulate genes essential on various metabolic processes and cell differentiation, but also exert anti-inflammatory properties after brain injury or neurodegenerative diseases **(Yonutas and Sullivan, 2013)**.

PPAR-γ is expressed in various cell types; adipose tissues, muscles, brain, and immune cells. PPAR-γ is involved in the expression of many genes and contributes to glucose homeostasis, insulin sensitivity, lipid metabolism, immune responses, inflammation, and cell fate **(Desvergne and Wahli, 1999)**. Also, PPAR-γ activation plays a crucial role in the regulation of proliferation, metabolism, differentiation, development, and inflammatory responses of the CNS **(Gurley et al. 2008)**, in this way PPAR-γ agonists have significant therapeutic potential in brain disorders **(Villapol, 2018)**. PPAR-γ plays effective neuroprotective role in the peripheral and brain inflammation, and PPAR-γ agonist in the regulation of neuroinflammatory processes following brain injuries. Also, its role in apoptosis, neurogenesis, differentiation, and angiogenesis that are triggered as consequence of brain damage **(Villapol, 2018)**. Three PPARs isoforms found in neurons and glial brain cells and support the role of these nuclear receptors in neuroprotection and in cognition and behavior **(Agarwal et al., 2017)**.

In cells that express an APC-containing destruction complex, activation of PPAR-γ induces CPD **(Fig. 4)**. Thiazolidinediones (TZDs), a class of PPAR-γ agonists, induce a reduction in the cytoplasmic level of β-catenin in both adipocytes **(Gerhold et al., 2002)** and hepatocytes **(Sharma et al., 2004)**.

PPAR-γ is down-regulated while the canonical Wnt/β-catenin pathway is up-regulated **(Fig. 5) (Takada et al., 2009)**. PPAR-γ inhibited Wnt signaling in adipogenesis and kidney diseases, partly through downregulating the levels of β-catenin **(Lee and Han, 2010)**. Wnt/β-catenin pathway and PPAR-γ interact through a TCF/LEF β-catenin domain and a catenin-binding domain within PPAR-γ **(Fig. 4) (Lu and Carson, 2010)**. PPAR-γ agonists can act as neuroprotective agents and promoting synaptic plasticity through a Wnt/β-catenin/PI3K/Akt pathway interaction **(Fig. 4) (Farshbaf et al., 2014)**.

In several diseases, β-catenin signaling inhibits PPAR-γ **(Kumar et al., 2014)**. Troglitazone, a PPAR-γ agonist, decreased c-Myc levels **(Akinyeke and Stewart, 2011)**. In intestinal fibrosis, the activation of Wnt/β-catenin observed and the use of PPAR-γ agonist inhibited Wnt/β-catenin pathway activation **(Di Gregorio et al., 2014)**. In several diseases, PPAR-γ is upregulated while canonical Wnt/β-catenin is downregulated; in arrhythmogenic right ventricular cardiomyopathy, osteoporosis, and certain neurodegenerative diseases; Alzheimer's disease; bipolar disorder, and schizophrenia **(Lecarpentier and Vall´ee, 2016)**. Conversely, in other diseases, PPAR-γ is downregulated while canonical Wnt/β-catenin is upregulated; in type 2 diabetes, cancers, and certain neurodegenerative diseases (amyotrophic lateral sclerosis) **(Lecarpentier and Vall´ee, 2016)**. PPAR-γ activation selectively decreased PDK mRNA **(Lecarpentier et al., 2017)**.

3. Conclusion

Canonical Wnt/β-Catenin pathways are considered as an important contributor to the induction of autism via VPA which confirmed that autism ASD is believed to be a result of interactions between genetic and environmental factors. Therefore, inhibition of Wnt/β-Catenin cascade pathways through inhibition of LDHA, PDK, c-Myc with GLUT-1 as well as enhancement of PPAR-γ, PTEN and increasing the level of ACh neurotransmitter in brain tissues would have a protective effect against VPA-induced autism. This effect would be reflected on improving of the major core behaviors characterized for autism; sociability and social preference, enhancing stereotypic behaviors, decreasing hyperlocomotion activity with significant improvement of histopathological features of the brain.

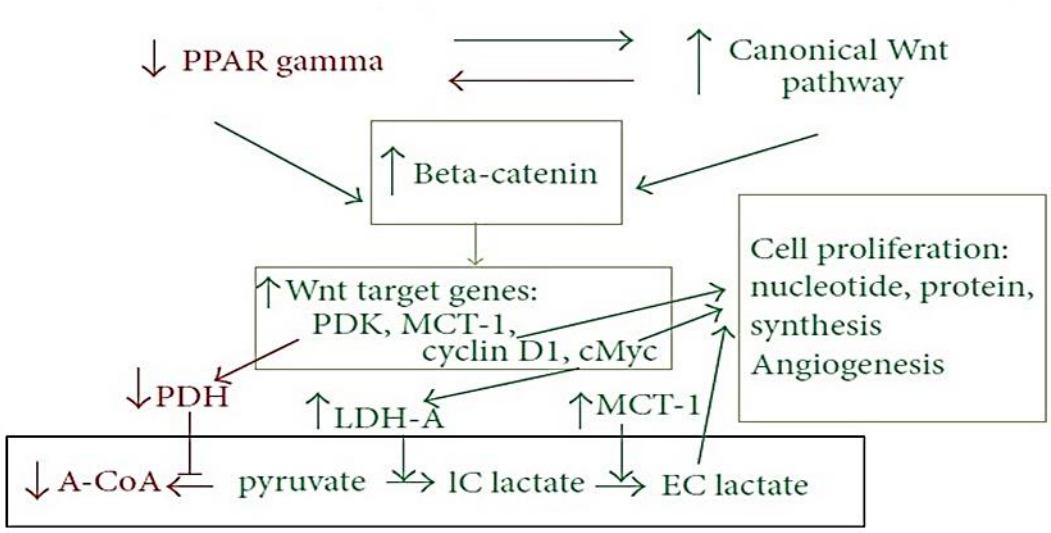

Figure 5: Interactions between PPAR-γ and the canonical Wnt/β-catenin pathway. Green arrow: activation; red arrow: inhibition; A-CoA: acetyl-CoA; GK: glucokinase; IC lactate: intracellular lactate; EC lactate: extracellular lactate; LDHA: lactate dehydrogenase- A; MCT-1: monocarboxylate lactate transporter-1; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; PPAR-γ: peroxisome proliferator-activated receptor-γ.

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