Review article

A systematic review on Valproate induced rat model of autism: Pathophysiology, treatment, developmental and neurobehavioral assessment of rat offspringsKavitha U.¹, Kayalvizhi E.¹, Revathy K.², T.R.Brindha³, Muthulakshmi R.¹, Chandrasekar M.⁴¹Meenakshi Medical College Hospital & Research Institute, Kanchipuram, Tamil Nadu, India²Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India³Vel R.S Medical College, Chennai, Tamil Nadu, India⁴Bharath University of Higher Education, Chennai, Tamil Nadu, India

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Corresponding author: **Kayalvizhi E.** Email: kayalgkbs@gmail.com**ABSTRACT**

Autism Spectrum disorder (ASD) is characterised by certain degrees of disabilities in social communication, restrictive repetitive behaviour and altered motor and sensory perception. Translational research is carried out by creating animal models of autism to find out the correlation between behavioural changes and the pathology of brain tissue and for clinical trials of newer therapeutic formulations. Valproate induced animal model of autism has significant validity to demonstrate ASD manifestations. An early appreciation of ASD symptoms is needed for a better prognosis. So assessment of behavioural abnormalities and development stones in the first month of animal life are much important to study ASD. An extensive literature search was done on different databases. (PUBMED-MeSH, PMC, Web of Science, Google scholar and Research Gate). Original articles reported between the years 2000-2020 were selected. PRISMA protocol was followed. There are good quantities of studies on behavioural assessment of valproate induced animal model. This review explains the pathophysiology and various treatment modalities tried in valproate induced animal model and it also enlists the developmental and behavioural assessment methods of rat off springs. It will be useful to demonstrate all signs of autism.

Keywords: Autism spectrum disorder; valproate induced animal model; neurobehaviour; neurodevelopmental reflex.

INTRODUCTION

Autism spectrum disorder (ASD) is the neurodevelopmental disorder in which the affected individuals exhibit a different range of behavioural abnormalities like social impairment, poor communication skills, and repetitive behaviour. In rural and urban settings, the percentage prevalence of ASD is around 0.11 and 0.09 in children aged 0-15 years respectively (1). Genetics and the environment play an important role in the normal development of the brain. Behavioural disorders may also be due to a combination of contributions from the environment and genetics. Physiology of regeneration of the nervous system is a very complicated process; any small alteration in the nervous system during the developing stage is expected to be irreversible. In addition to that, diagnosis is delayed due to lack of awareness. So the treatment of ASD remains challenging.

Translational research is carried out by creating animal models of Autism. There are different animal models of autism which can be categorized into single-gene mutants, epigenetic factors/ prenatal exposure of chemicals, neonatal lesions of brain areas and other genetic diseases associated with autism (2). Mouse models of human neuropsychiatric diseases are designed to optimize i) face validity, i.e., resemblance to the human symptoms; ii) construct validity, i.e., similarity to the underlying causes of

the disease; and iii) predictive validity, i.e., expected responses to treatments that are effective in the human disease (3).

Valproate exposure at the first trimester of pregnancy in child (4) and 12th embryonic day of rat offspring (5) i.e. around the time of neural tube closure and formation of brain stem nuclei has strong evidence and validity for the development of autism. This model showed anatomical, behavioural, pathological and etiological similarities with human system. Valproate induced autism animal exhibited following autism like behaviour i) sensory perception is extreme at both ends, these rats had lower sensitivity to pain and higher sensitivity to non painful stimuli, ii) diminished acoustic prepulse inhibition, iii) locomotor and repetitive/stereotypic-like hyperactivity combined with lower exploratory activity, iv) decreased flexibility to change strategy: social approach; and place preference to conspecifics and v) decreased number of social behaviors and increased latency to social behaviors. Autism induced rats showed delayed performance in olfactory discrimination test and negative geotaxis (6).

The initial step in management is early diagnosis of ASD. Neurobehavioural tests which assess the different domains are necessary for appreciation of ASD. Rat offspring exposed to VPA 500 mg/kg

exhibited some physical abnormalities including shorter snouts, multiple toes, or dwarfism and tail malformation were also observed in autism induced rats (7). Pathophysiology of ASD and effects of VPA on brain development were well studied. VPA mainly creates imbalance in excitatory and inhibitory neurotransmitters additional to other effects on brain development which may be the important factor for behavioural abnormalities. This review was aimed to explore the knowledge about various behavioral assessment methods along with developmental stones at different ages of young rat off springs during the first month of postnatal period (before sexual maturity). It will guide the researcher to demonstrate all signs of ASD and will be helpful to do clinical trials by using different therapeutic drugs.

OBJECTIVES

1. To study pathophysiology of valproate induced animal model of autism

2. To list the criteria for maternal and child health of valproate exposed rats and its offsprings
3. To describe the developmental stages and milestones of rat off springs
4. To discuss the neurobehavioral assessments based on clinical features

MATERIALS AND METHODS

Design: PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-analyses. Its checklist and 4 phase flow diagram was used to demonstrate the quality of the review. The screening was done and Research studies focused only on rat off springs were included and others were excluded. Original articles that have been done on animal models of autism and neurobehavioural assessment tests for rat offsprings were searched in the database of PUBMED-MeSH, Google Scholar, Science Direct, and Web of science. Published articles between the year 2000-2020 and full text articles were selected for review.

Table 1: Database search

Database	Year	Search statergy	No of articles
NCBI PUBMED	2000-2020 Date of search June 2020	((("Autism Spectrum Disorder"[Mesh]) AND "Models, Animal"[Mesh]) AND "Behavior, Animal"[Mesh])	n=369 abstracts

Fig. 1: PRISMA flow chart

Valproate induced animal model of autism

Rodent model of autism was created by exposure of rat fetuses to valproic acid (VPA) on the 12.5th day of gestation at the dose of 600mg/kg. Influence of gender on valproate induced model was studied in which male rats showed lower sensitivity to pain, increased repetitive/stereotypic-like activity, higher anxiety, decreased level of social interaction, female VPA rats exhibited only increased

repetitive/stereotypic-like activity which suggests that they are gender specific (8).

The validity and efficacy of this model and appearance of ASD symptoms was demonstrated by administering VPA at different intervals and doses. VPA administered i.p. at 500 mg/kg on E12.5 is less toxic than at 600 mg/kg in terms of fetal survival and pregnancy outcome (9). The maternal and child health effects of prenatal valproic acid exposure at different interval for inducing autism

from embryonic day 7 to embryonic day 15 in Sprague–Dawley rats were studied (10). VPA exposure at 9th day of embryonic life reduced the number of live birth and at 12th day of embryonic life exposure produced significant ASD symptoms (11). TuJ1 – indicator of immature neurons of embryo, development of TuJ1 was impaired in valproate exposed supplier group (pregnant rats purchased from outside) than valproate exposed pregnant rats of same animal house. This indicates that validity of VPA animal model is also affected by maternal factors including shipping stress (movement from one animal house to another place) and environmental change during early pregnancy (11).

Pathophysiology of behavioural changes in Valproate induced ASD rats

The pathology of autism needs to be analysed in order to reverse the changes and produce better outcomes. Studies on human beings are conducted by using brain autopsy of autism patients. In Autism induced rats, electrophysiological recordings of lateral nucleus (LA) of amygdala showed hyper excitability and enhanced long term potentiation which is associated with the presynaptic efficiency of excitatory synaptic transmission. Autism like behaviour is mainly due to the disruption of the synaptic excitatory/inhibitory (E/I) balances in the LA (12). The level of glutamatergic/ GABAergic proteins determines the Excitatory and inhibitory balance. There is increase expression of glutamatergic proteins in postnatal brain of offspring, this is due to influence of VPA on transcription factor Pax6 (13). VPA related to ASD pathology is also explained as early brain overgrowth and increased network excitability that is due to histone acetylation and methylation which involves in gene regulation. When there is increase number of neurons and dendrites, hyper connectivity, and hyper excitability the balance (excitatory/inhibitory) gets altered results in behavioural abnormalities.

VPA exposed rats at the embryonic stage exhibit reduced synthesis of serum PUFAs due to the down-regulation of liver metabolic enzymes, which results in nervous system injury and ASD related behavioral changes (14). Hyperserotonemia in pregnancy due to Valproate /SSRI treatment might also be one of the reasons of the increasing frequency of autism (15). Anandamide- a neurochemical produced naturally in brain and it is responsible for motivation and pleasure feeling. Anandamide acts through (CB1 receptor) cannabinoid receptors; phosphorylation of this receptor indicates its activity. Rats prenatally exposed to VPA disturb phosphorylation of CB1 cannabinoid receptors in the amygdala, hippocampus and dorsal striatum (16). Fatty acid amide hydrolase (FAAH) is a degrading enzyme of Anandamide; FAAH was increased in VPA exposed rat offsprings during infancy period (17). The social motivation impairment

may be due to dysfunction of the dopaminergic–oxytocinergic vmPFC–VS–amygdala brain network (18).

Therapeutic modalities tried in valproate induced autism and its behavioural assessment

Postnatal environmental manipulations – Enrich environment to valproate exposed autism reversed the ASD symptoms like decrease anxiety and repetitive behaviour and increased social communication (19). From postnatal day 14-40, daily dose of 300mg/kg Green tea extract ameliorated the behavioural aberrations in postnatal valproate induced rat off springs (20). Treatment with B.monniere (300mg/kg/po) from PND 21-35 produced better behavioural pattern in prenatal valproate induced autism rats. (21). Docosahexaenoic acid (DHA) (75, 150 or 300 mg/kg/day) for 21 days play a significant neuroprotective role in hippocampal neuronal cell and ameliorates dysfunctions in learning and memory in valproate (600mg/kg) rat autism model (22). Korean red ginseng treatment reversed abnormal locomotor activity and sensitivity to electric shock to control level in valporic acid (VPA) induced model (23). Valproic acid (600mg/kg) induced autistic-like behaviors like increased pain threshold and impaired social interaction in male rats was reversed by resveratrol administration (3.6 mg/kg, s.c.) (24). Fenofibrate (FBR), a peroxisome proliferator-activated receptor α (PPAR α) agonist, improved behaviour pattern in social interaction and communication in rats prenatally exposed to VPA. Long-term FBR treatment started at weaning and continued until young adulthood has positive effects on social behavior. Stress-induced motivational anhedonia is relieved by repeated treatment with FBR. Its effect could be related to the modulation of mesolimbic dopaminergic transmission (25).

Group of Pregnant rats exposed to valproate along with oil extract of *Nigella sativa* (600mg/kg/po) and valproate with folic acid (400ug) had positive effects of improving the ASD features (26). Zinc (20mg/kg/sc) along with VPA administration showed reduced autistic like symptoms (27). Astaxanthin (2 mg/kg) was given on PND 25 for 4 weeks, improved behavioural pattern in VPA exposed rat offspring it might be due to its anti- oxidant activity (28). Betaine- a methyl donor reduces the serum homocysteine concentration and it could ameliorate the autism like behaviour (29).

Assessment Methods

1. Assessment of Maternal and Child health of Rat off springs (30).

To ensure the maternal and child health following parameters can be assessed on particular days of postnatal period.

Table 2: Assessment Criteria and Postnatal Days

Assessment Criteria	Postnatal Days
Litter size	PND 0
Postpartum mortality	PND 1to PND 4
Mortality during lactation	PND 5to PND 22

Litter survival rate was calculated by determining the ratio of the total number of pups that reached the weaning age to total number of pups born per litter.

2. Stages of rats from birth to Adult (31).

Based on Neuronal development and behavioural response, the first month of rat's life was divided into five periods until sexual maturity.

Fig. 2: Stages of rat's lifecycle

3. Assessment of developmental stones of rat offsprings (32).

Body weight and length (33) are measured along with developmental stones as the indicators of postnatal

maturation. Rat litter's birth weight can be measured at PND1 and weight gain should be monitored by checking body weight at regular intervals. Valporate treated rat offsprings showed low weight (4) after 2 week of postnatal period when compared to control, this finding was contradictory to other study in which there was no significant weight(5) changes between the groups.

Table 3: Developmental stones and postnatal days

Developmental Stones	Average Postnatal Days to appear
Ear unfolding	PND 2
Pinna detachment	PND 2-5
Incisor eruption	PND 6-7 & PND 10
Fur appearing	PND 9
Eye opening day	PND 13-16
Ear opening day	PND 12-16

4. Neurodevelopmental Assessment of Young rats (upto 30 days/ before sexual maturity)

4a. Neurodevelopmental Reflexes

Appreciation of neurodevelopmental reflexes in the first 2 weeks of rats life period are the best assessment tools at which complex behaviour testing is not possible (34).

Table 4: Sensory and motor response tests

Name of the test	Parameters to be tested	Age of rat offsprings at reflex appears	Description of test
Forelimb Grip Test	Neuromuscular junction	PND3	Stimulation of palm of forepaw results in flexion of all digits.
Hind limb Grip	Neuromuscular junction	PND 3	Stimulation of sole of hindpaw results in flexion of all digits.
Surface righting reflex	Non-spatial sensory perception	PND 3, PND 4	Pup's ability to turn over from supine position.
Negative geotaxis	Spatial sensory perception	PND 7	Pup's ability to turn 180° on a 25°/30° inclined placed head down (35).
Cliff avoidance test	Spatial sensory perception it also assess the integration of exteroceptive input and loco-motor output	PND 9	The time that the pup spent to move away from the "cliff", when it placed with its nose and foreleg over the edge of a wooden platform (30 cm in height)
Nociception/Tail flick test	Non-spatial sensory perception	PND 10	The latency that the pups flicked its tail to intense radiant heat was recorded.
Startle reflexes	sensorimotor reaction	PND 10	Jerk movement/ startle response when loud noise is presented.
Dynamic air righting	Non-spatial sensory perception	PND 14	The pup's ability to turn over in the air from supine position and fall down onto 4 limbs.

4b. Olfactory discrimination test: PND 9- 11

The time taken by the animal to identify home bedding through olfactory cues was tested. Animal

placed on a central platform with its own home bed and new bed on either side. The latency (in seconds) to reach the nest odor was significantly increased in

Valproate treated animals when compared to controls (36). Sense of olfaction is crucial for the development of social behavior and social recognition, but exposed to VPA alters the olfaction.

4c. Sucrose consumption test (25)

Sucrose solution 2% (w/v) and tap water were placed to rats in two different bottles free of choice. Irrespective of bottles position the preference for sucrose was calculated as percent volume of sucrose solution over total liquid volume consumed. The dopaminergic signalling underlying reward responses is impaired by VPA exposure, this was demonstrated by decreased sucrose preference response.

4d. Test for motor coordination: Rotarod Test

PND 20-22

Rodents are placed on the rotating rod at the particular speed (rpm). The duration to maintain the balance in the rotating rod without falling was noted..

4e. Test for memory (Attention) - PND 21-23

Novel object recognition test: Rodents were exposed to novel and familiar objects at the time. The time spent with novel objects when compared to familiar is noted. It evaluates cognition, particularly recognition memory (36).

T maze/Y maze: Animals are allowed to explore the arms of T and Y maze with one arm closed. While testing all three arms of maze are opened, the time spent in newly opened arm when compared to other two arms is noted.

Radial arm maze: PND 26-28 days. There are 8 arms to increase complexity of task. It tests reference, spatial and working memory. As positive reinforcement food is used. No of errors in remembering the arms of the maze that is visited previously to find the food reward (37).

4f. Tests for exploratory behaviour- oddity discrimination test: PND 21-23

After habituation for two days to the test area, 3 identical and 1 odd object are given. Object examination is recorded for 5 minutes (37).

4g. Tests for stereotypic and compulsive-like behaviors

Marble burying test: PND 27-31 test is used for the assessment of repetitive behavior. Animal's attitude towards the black marbles when placed on the cage is assessed. The time until over 75% of the marbles are buried is recorded (38).

Nestlet Shredding Test: Animal is placed along with preweighed nestle material for 30 min in the cage. After 30 min, percentage of nestlet shredded is calculated as ratio between preweighed nestlet and weight of remaining unshredded nestlet (39).

T/Y arm maze: Instead of spontaneous alternation behaviour in T maze/Y maze, animal prefers the same arm indicates repetitive behaviour.

Hole board test: Dipping behavior was scored by the number of times an animal inserted its head into a hole and latency for first dipping (25).

4h. Evaluation of social interaction

Ultrasonic vocalisation test: This test is being sensitive to social context; these neonatal calls may help to reveal reduced social attachment or abnormal processing of social information. USVs emitted during isolation by male and female rat pups prenatally exposed to either valproic acid (VPA) demonstrated early sociocommunicative deficits. Around PND 6-9 the pups in sound-isolated box vocalization were recorded for 3 min with an ultrasound microphone and preamplifier. Ultrasonic calls were quantified using software (40).

The social test chamber. Three Chamber test. Animals are habituated in the center compartment. While testing new animal was provided in one chamber and other chamber is left empty. The time spent with mouse chamber and empty chamber are compared (41). In the test of preference for social novelty, a significant propensity to spend more time with a new mouse than with a familiar mouse also observed. Sociability function mainly relates the physiology of striatum, posterior cingulate, premotor cortex, amygdala, nucleus accumbens and anterior cingulate cortex.

4i. Tests for anxiety behaviour (36)

Chromodacryorrhea is a condition found in rats due to porphyrin over-production by the Harderian glands in the eye. Chromodacryorrhea is the indicator for general stress, it is nothing but red crust formation near eyes, nose.

Elevated plus maze: The time spent in the enclosed arms of the maze and the numbers of crossings from the enclosed to open arms were recorded. When compared to control group VPA males spend less time in open arms and longer time in the safer closed arms of the maze indicates VPA exposed male rats were more anxious (36).

Open field maze: Rat offspring prenatally exposed to VPA showed higher sensitivity to electric shock seizure and increased locomotor activity in open-field test. Two indicators of emotionality such as locomotor activity and defecation can be assessed from OFM. Rearing, total distance crossed and time spent in inner, outer squares are recorded for local motor activity. Time spent in outer and Inner Square: Duration spent in outer zone/Thigmotaxis or wall-hugging behavior is indicative of amplified anxiety-related behaviour. Frequency of rearing and defecation are the (42).

CONCLUSION

This extensive review explains about the methodology, pathophysiology of VPA induced animal models and different treatment modalities that tried to reverse symptoms of ASD. The main scope of this review is focused on various assessment methods for developmental stones and neurobehaviour at different postnatal days of rat offspring. This knowledge will give important clues about the areas to be considered when doing research on valproate induced animal models of autism. More studies are needed to exactly map the cause- effect relationship with prevention and treatment of ASD.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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