Original Article

The Impact of Sitagliptin on Sodium Valproate-Induced Autism in a Mouse Model

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Abstract

Background: Autism spectrum disorder (ASD), a neurodevelopmental disease, is described by problems with social interaction and communication that arise at an early age. The only approved drugs for the treatment of ASD are risperidone and aripiprazole. **Objectives:** The aim of the article is to explore the potential therapeutic effects of sitagliptin on the induced offspring model of autism. Also, to evaluate the effect of sitagliptin on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). **Materials and Methods:** We induced the disorder in mice by injecting pregnant mice with sodium valproate (600 mg/kg). Prenatal sodium valproate–exposed mice were split into four different groups, with two experimental groups taking sitagliptin (10 mg/kg and 15 mg/kg) and risperidone (1 mg/kg), and a control group receiving normal saline. Behavioral tests, including social interaction assessments were divided into three phases: habituation, familiarization, and testing, and lasted for 15 min, were conducted on postnatal day 65; also, anti-inflammatory marker assessments like TNF-α and IL-6 were conducted on postnatal day 66. **Results:** The study found that sitagliptin significantly improved behavioral disorders (social communication) and reduced neuro-inflammation in the brain. Sitagliptin therapy forcefully enhanced the cognitive function of ASD mice by regulating neurogenesis that could be connected with the powerful antioxidant and anti-inflammatory actions that sitagliptin possesses. **Conclusion:** Sitagliptin showed potent anxiolytic and anti-inflammatory properties that improved behavioral activities in the mice. These findings suggest that sitagliptin could be promising a potential treatment option for individuals with ASD.

Keywords: Autism, risperidone, sitagliptin, sodium valproate

Introduction

Autism spectrum disorders, abbreviated as ASD, are a group of complex illnesses brought on by a junction of epigenetic, physical, and genetic factors during early prenatal and postnatal infancy. These factors can have an effect on the development of the child both before and after birth.[1]

2013's Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, states that the criteria for diagnosing ASD are solely behavioral. Hallmarks of ASD include deficiencies in interpersonal relationships and communication, in addition to compulsive behavior, attention deficits, and behavioral inflexibility.[2]

A mixture of hereditary components and environmental variables is frequently used to define it. Hereditary factors that cause ASD include genes linked to cognitive impairments, neurological illnesses, common pathways, ASD, DNA mutations, and environmental factors that affect gene expression and protein function.[3]

ASD inflammation may be brought on by disequilibrium between cytokines that promote inflammation and those that reduce it caused by a low quantity of anti-inflammatory cytokines. Infection during pregnancy may produce inflammatory markers that go over the placenta and cause inflammation of the fetus's neurons. The oxidative stress, neuropathy, as well as metabolic

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_398_23

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Submission: 05-Apr-2023 **Accepted:** 22-May-2023 **Published:** 24-Sep-2024

How to cite this article: Abbas AS, Selman SM, Mahrath AJ. The impact of sitagliptin on sodium valproate-induced autism in a mouse model. Med J Babylon 2024;21:544-50.

abnormalities may all have an impact on the genesis of ASD.[4]

Individualized, intensive treatments incorporating cognitive and behavioral components have proven to be the most effective treatments for ASD.[5] Despite the significant expenses of patient care, there is currently no known treatment for ASD; instead, treatments mostly target the disorder's symptoms. As a result, developing interventions to prevent ASD must be given top attention, along with encouraging a thorough inquiry into its pathophysiological mechanisms.[6] The only two pharmaceutical therapies recognized by the FDA for managing ASD symptoms are risperidone and aripiprazole. Both antipsychotic medications are used to treat aggression and irritability.[7]

The dipeptidyl peptidase IV (DPP-4) enzyme is inhibited by sitagliptin, which is a competitive inhibitor. It is possible to administer it in doses that reduce the measured activity of DPP-4 for a period of 12 h by more than 95%. Increased insulin secretion, decreased glucagon levels, and reductions in both postprandial and fasting hyperglycemia are related to this phenomenon. When administered as a monotherapy in patients with type 2 diabetes, the drug sitagliptin lowers HbA1c by an average of roughly 0.8%.[8]

Animal models are recommended for the investigation of ASD, and it is recommended that these models use a multimodal approach that considers the behavioral, neuropathological, physiological, and hereditary aspects of ASD. The mice that carry a mutation in any of the several ASD risk genes are used to create animal models the vast majority of the time.[9] Although rats exhibit a significantly increased indication of social contact when compared to mice, which is the rodent model that is most commonly used, some researchers are beginning to emphasize the value of comparing mouse and rat rodent models and the potential for cross-species convergence and divergence.[10]

Since prior research indicated that sitagliptin had potent antioxidant and anti-inflammatory effects on Alzheimer, Parkinson, and dementia illnesses, it was utilized in this investigation as a powerful neuroprotective drug among other members of the DPP-4 group.

Materials and Methods

Experimental animals

The research involved 60 healthy adult albino mice, comprising 40 females and 20 males, with a weight range of 26–39 g. These mice were acquired from the Ministry of Science and Technology in Baghdad, Iraq. They were kept in standard plastic cages in the animal house of the College of Medicine, Babylon University. The mice were housed under optimal conditions, with a room temperature of 24 ± 5°C, humidity levels of 65 ± 5%, and a 12-h light/dark cycle. They were given unlimited access to food pellets and water.

Mating and vaginal smear

In this study, the adult mice were separated into 20 mating groups, each consisting of 1 male and 2 females. To confirm successful mating, the females were examined for the presence of a vaginal plug, composed of coagulated secretions from the male's vesicular and coagulating glands. The plug can fill the female's vagina and usually lasts 8–24 h after breeding. The female was lifted by her tail and peered inside her vaginal opening for a whitish mass to find the plug before lowering her.

To determine day zero of pregnancy, vaginal swabs were collected using a cotton-tipped swab after gently stretching the vaginal opening. The collected specimen was smeared onto a new glass microscope slide and air-dried. The slide was then examined under a microscope to identify the presence of sperm [Figure 1], which indicates that day zero had occurred for the sampled mouse.

Preparation of animals and induction of autism

On the 12th day, the pregnant female animals were isolated into two groups. The control groups—10 a group with 2 pregnant female mice each—received simply water intraperitoneally (i.p.). Each of the 10 experimental groups, including 2 pregnant female mice, received a single intraperitoneal injection of 600 mg/kg sodium valproate (Depakine®) to create the autism model.[11]

Preparation of drugs

Sodium valproate salt, sitagliptin, and risperidone were used as a tablet dosage form from Sanofi, MSD, and Janssen companies. Tablets were broken up into powder using a mortar and pestle. The powder of sodium valproate was subsequently mixed in water for injection at concentration of 200 mg/2 mL, and a single injection of

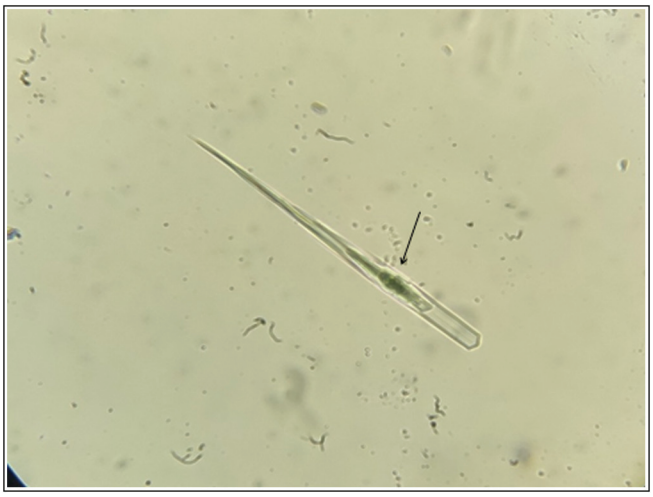

Figure 1: Identification of mouse sperm by light microscope

600 mg/kg body weight was given intraperitoneally.[11] The resulting powder of sitagliptin was dissolved in water at strength of 1 mg/10 mL. The powder was then delivered orally at a dosage of 10 mg/kg[12] and 15 mg/kg body weight for 20 days. Risperidone powder was dissolved in water at 1 mg/10 mL. The powder was then given orally for 20 days at 1 mg/kg body weight.[13]

Experimental design

The offspring mice were separated from the mothers on the 40th postnatal day and divided into 8 groups, each of which consisting of 10 animals:

- The offspring in the control groups were given the following treatments orally: group 1 received normal saline, group 2 obtained sitagliptin 10 mg/kg, group 3 obtained sitagliptin 15 mg/kg, and group 4 got risperidone 1 mg/kg.
- VPA-induced offspring groups: group 5 obtained normal saline by oral administration, group 6 received sitagliptin 10 mg/kg orally, group 7 got sitagliptin 15 mg/kg orally administered, and group 8 obtained risperidone 1 mg/kg via oral administration.

Social interaction test

Social interaction was examined using a rectangular apparatus consisting of three connected compartments, each measuring 20 × 30 × 30 cm. Access to each compartment was provided through one of two holes, each measuring 10 × 7 cm. The central chamber was used as the initial beginning point for each phase of the test. The social interaction test was divided into three phases: habituation, familiarization, and testing, and lasted for 15 min.

In phase I (habitation), which lasted for 5 min, the experimental mouse was placed in the central compartment. The doors to the other two compartments were locked, allowing the mouse to explore the environment freely.

In phase II (familiarization), which also lasted for 5 min, the experimental mouse remained in the central compartment. The doors to the other two chambers were left open, and no other mouse was introduced. The researchers noted the number of times the mouse moved between the three compartments [Figure 2].

In phase III (5 min), each side chambers contained a wire cage with a diameter of 10 cm. An unfamiliar mouse was placed in the wire cage on the right side, while the cage on the left was left empty. After being placed in the central compartment, the experimental mouse could explore all three compartments. The researchers observed and recorded the interaction between the two mice [Figure 3].

Mice decapitation

After a series of behavioral tests, the mouse was beheaded 24 h after its final therapeutic dose as a sacrifice. Mouse heads were sliced with surgical shears. Cutting the skin on top of the head revealed the skull, which was then flipped around to catch it. A scalpel cut the skull horizontally. The individual saw their brain after forceps removed the skull. After carefully removing any nerves that were holding the brain in place, the procedure began with the olfactory bulbs and continued with the removal of the brain itself. After removing the olfactory bulbs and cerebellum, just two brain hemispheres were removed. The two hemispheres were rinsed with 10% (w/v) phosphate-buffered saline (PBS) (30 mmol/L, pH 7.0) and placed in a flat tube with ice to lower the brain's temperature as rapidly as feasible. The refrigerator cooled the tube to −20°C. One gram of the frozen brain tissues was weighed and added to the Eppendorf tube containing PBS, then homogenized via homogenizer and rotated at 4°C for 30 s.

Figure 2: The movement of the mouse from chamber to chamber in social interaction test

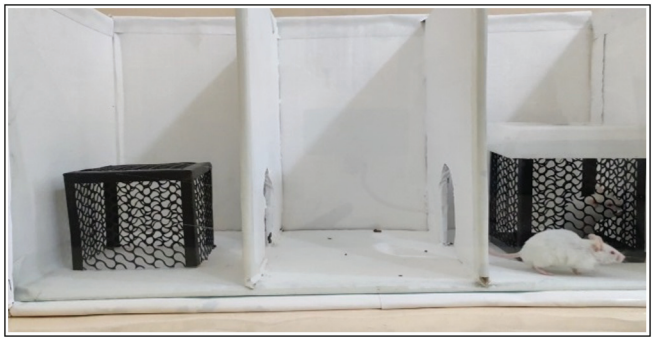

Figure 3: Experimental mouse spending time with strange mouse in social interaction test

Anti-inflammatory estimation

Using an enzyme-linked immunosorbent assay kit, the IL-6 and TNF-α concentrations in the tissue were determined.[14]

Statistical analysis

Statistical Package for the Social Sciences 26 was used for data processing, input, and analysis (SPSS). The study used a one-way analysis of variance and *post-hoc* least significant difference testing. Mean and standard deviation were shown (LSD). All of the above statistical tests were done with a significance level (*P* value) of 5%. A *P* value of

>0.05 indicates that the results are insignificant, whereas a *P* value of <0.05 shows significance. To be significant, the *P* value should be low.

Results

Social interaction test

Number of transitions between chambers

In contrast to the other groups, the number of transitions between chambers significantly increased ($P < 0.001$) in the low-dose control-sitagliptin group. Conversely, the number of transitions significantly decreased ($P < 0.001$) in the VPA-saline group as opposed to the other groups. The VPA-risperidone group exhibited a significantly higher number of transitions ($P < 0.001$) compared to the VPA-saline group. The high- and low-dose VPA-sitagliptin groups showed significant decreases in the number of transitions ($P = 0.001$ and $P = 0.017$, respectively) in contrast to the VPA-risperidone group. These results were presented in Figure 4.

Time spent with strange mouse

The normal control-saline group showed a significant increase in the time spent with the strange mouse ($P < 0.001$) compared to the other groups. In contrast, the VPA-saline group showed a significant reduction in the

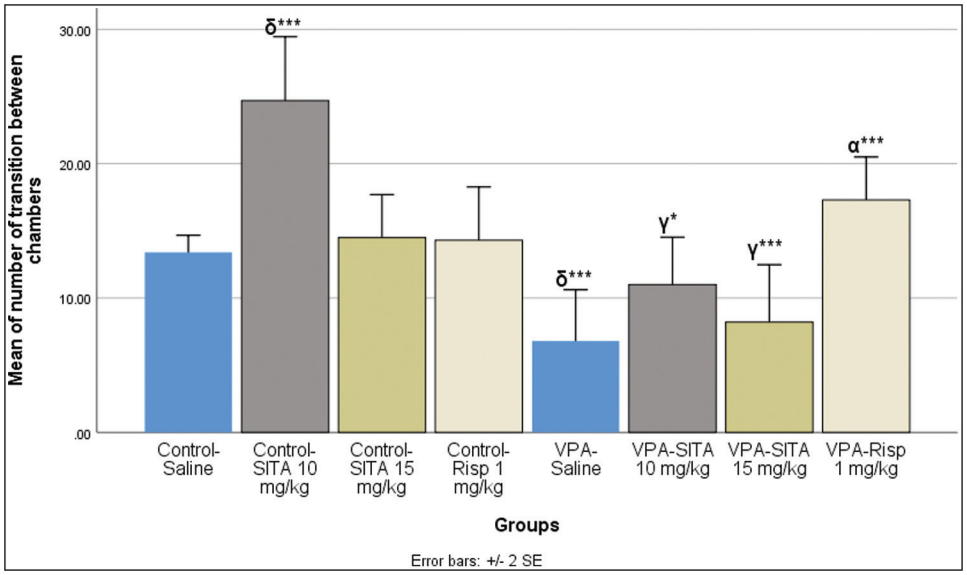

Figure 4: In the social interaction test, the effects of sitagliptin and risperidone on the total number of transitions between compartments. *$P \leq 0.05$ (significant); ***$P \leq 0.001$ (extremely significant). δ: As opposed with other groups; α: as compared to VPA-saline group, γ: in comparison with VPA-risperidone group

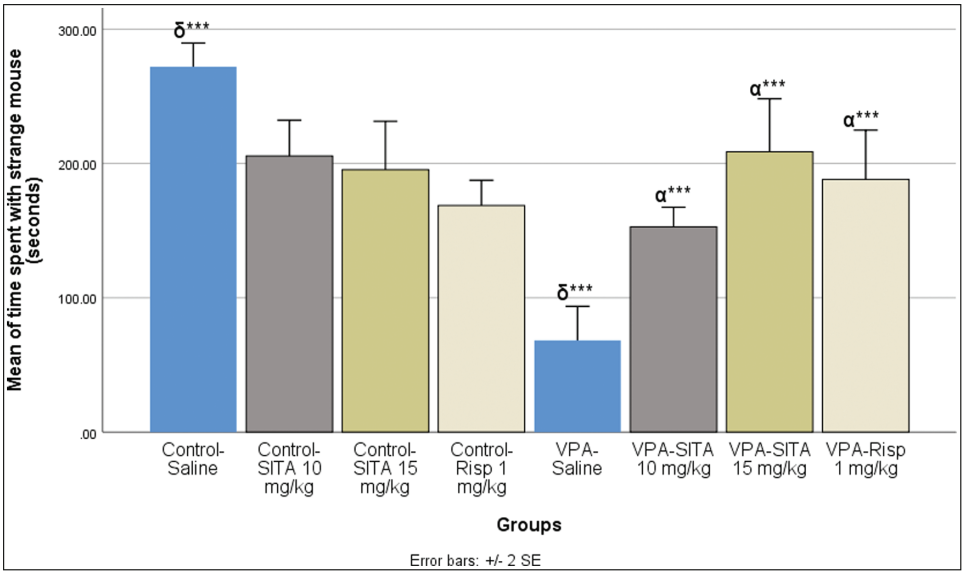

Figure 5: Effect of sitagliptin and risperidone on time spent with strange mouse in social interaction test. ***$P \leq 0.001$ (extremely significant). δ: As opposed to other groups; α: as opposed to VPA-saline group

time spent with the strange mouse ($P < 0.001$) compared to the other groups. On the other hand, both the low- and high-dose VPA-sitagliptin and VPA-risperidone groups exhibited a significant elevation in the time spent with the strange mouse ($P < 0.001$) in contrast to the VPA-saline group. Figure 5 presents all the aforementioned details.

Anti-inflammatory tests

Interleukin-6

The VPA-saline group exhibited a significant raising in the level of IL-6 ($P < 0.001$) in contrast to the other groups, while the VPA-risperidone group showed a significant decrease in the level of IL-6 ($P < 0.001$) in contrast to the other groups. The low- and high-dose VPA-sitagliptin and VPA-risperidone groups displayed a significant decrease in the level of IL-6 ($P < 0.001$) in contrast to the VPA-saline group. Additionally, the VPA-risperidone group exhibited a significant decrease in the level of IL-6 ($P < 0.001$) compared to both the low- and high-dose VPA-sitagliptin groups. The level of IL-6 was significantly reduced in the low ($P = 0.009$) and high ($P = 0.002$) doses control-sitagliptin groups compared to the control-saline group. These results were presented in Figure 6.

Tumor necrosis factor-alpha

The VPA-saline group had a significantly elevated level of TNF-α ($P \leq 0.001$) compared to other groups. Conversely, low and high doses of VPA-sitagliptin and VPA-risperidone groups showed an extremely significant decrease in TNF-α level ($P \leq 0.001$) in contrast to the VPA-saline group. In addition, both doses of control-sitagliptin ($P \leq 0.001$) and control-risperidone ($P = 0.019$) groups showed a significant decrease in TNF-α concentration in contrast to the control-saline group. The concentration of TNF-α in 10 mg/kg VPA-sitagliptin group was highly significantly increased ($P = 0.005$) as opposed to 15 mg/kg VPA-sitagliptin group and was extremely significantly elevated ($P \leq 0.001$) compared to the VPA-risperidone group. Details mentioned above are presented in Figure 7.

Discussion

This investigation, the first of its type in Iraq, is the first to look at the potential impact of sitagliptin on ASD. To create an animal model of autism, pregnant mice were injected with sodium valproate into their peritoneal cavity on gestational day 12. Mice that were exposed to sodium valproate during their prenatal development exhibited decreased sociability and social creativity as well as an increase in the release of inflammatory cytokines.

The lack of social interaction is a prominent symptom of ASD.[11] The results of this study indicated that mice offspring exposed to VPA had decreased social connections and exhibited signs of anxiety and fear, possibly due to an inability to understand signals from unfamiliar animals. However, treatment with sitagliptin and risperidone improved the poor social interaction in mice exposed to VPA.

Through the blood–brain barrier, insulin can attach to insulin substrate, mediating insulin-signaling pathways to control energy metabolism and protect neurons. Impaired insulin-signaling pathways could cause damage to the brain, which is suggested to be one of the etiologies of ASD.

Glucagon-like peptide-1 (GLP-1) binds and activates GLP-1 receptor (GLP-1R), regulating different neuronal

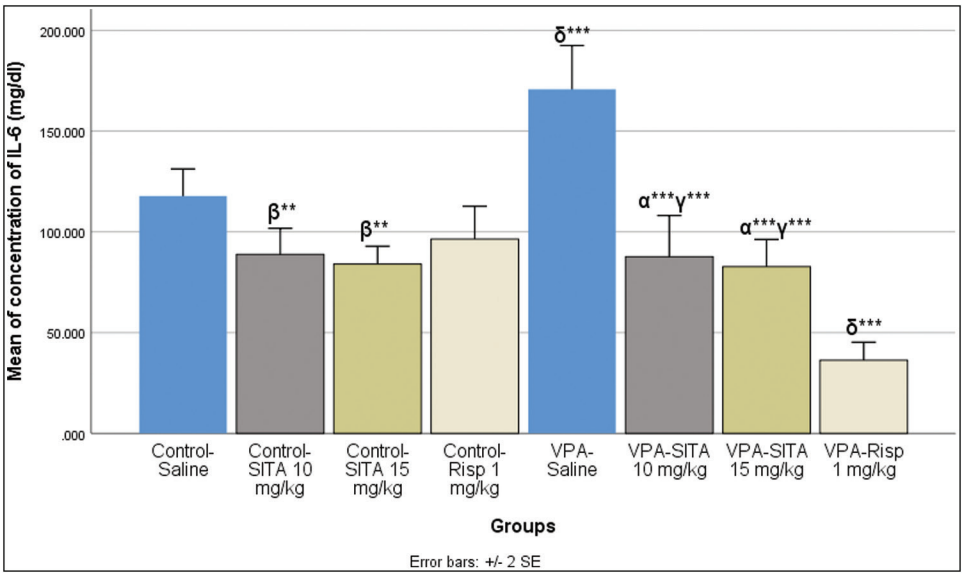

Figure 6: The influence of sitagliptin and risperidone on the amount of IL-6. $^{**}P \leq 0.01$ (highly significant); $^{***}P \leq 0.001$ (extremely significant). δ: as opposed to other groups. α: in comparison with VPA-saline group, β: as opposed to control-saline group, γ: in comparison with VPA-risperidone group

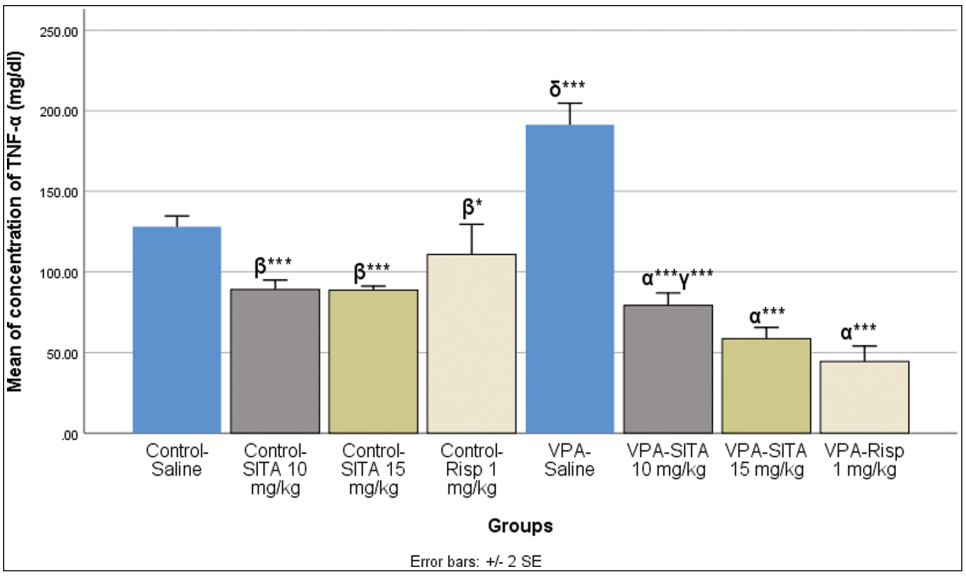

Figure 7: The influence of sitagliptin and risperidone on concentration of TNF-α. *$P \leq 0.05$ (significant); ***$P \leq 0.001$ (extremely significant). δ: As opposed to other groups; α: as opposed to VPA-saline group; β: as opposed to control-saline group; γ: as compared to VPA-risperidone group

functions. Sitagliptin attenuated neuronal apoptosis and preserved neurological function by stimulating GLP-1R, which is involved in activating insulin-signaling pathways.[15] GLP-1/GLP-1R signaling axis has recently been identified as a possible therapeutic target in CNS illness. GLP-1R was shown to be highly expressed in the CNS and within neurons. However, endogenous GLP-1 is rapidly broken down by DPP-4, shortening GLP-1's half-life.[15]

The study revealed that the administration of sitagliptin at doses of 10 and 15 mg/kg had a significant impact on enhancing the interaction time between stranger mice in the ASD mice model compared to the VPA-saline group. However, there was no substantial increase observed in the overall number of crossings between different compartments. Conversely, the administration of risperidone at a dose of 1 mg/kg demonstrated significant improvements in both tests by exerting its effects on dopamine receptors, particularly dopamine receptor D2, and serotonin receptors, specifically the 5-HT2A receptor. This study also highlighted the essential role of inhibiting these receptors in suppressing VPA-induced hyperlocomotion while promoting neuronal growth and cognitive parameters.[13,16] Furthermore, a comparison between the low dose of 10 mg/kg control-sitagliptin group and the control-saline group revealed a substantial increase in the number of transitions between chambers.

It is suggested that sitagliptin acts its roles, such as reducing blood glucose level, improving behavioral activities, anti-inflammatory activity, and anti-oxidative stress in a GLP-1/GLP-1R-dependent way.

The treatment of mice prenatally exposed to VPA with sitagliptin and risperidone resulted in a reduction in the concentrations of the inflammatory cytokines TNF-α and IL-6. The concentrations of TNF-α and IL-6 were significantly reduced in the VPA-risperidone group compared to the VPA-sitagliptin groups, and these amounts were also significantly declined in the 15-mg/kg sitagliptin group in contrast to the 10-mg/kg sitagliptin group. These results are consistent with those obtained from a past investigation on dementia,[12] which showed that the levels of pro-inflammatory cytokines were reduced with the use of sitagliptin.

The study found that sitagliptin has powerful anti-inflammatory effects at both the nuclear and cytoplasmic levels,[17] and the drug's effects on the innate immune system contributed to these properties. Sitagliptin has the potential to reduce the activation of NF-κB (nuclear factor kappa B) as well as cytokine expression.[18] The drug also significantly improved neurogenesis and neuronal plasticity and increased BDNF (brain-derived neurotrophic factor) expression, which is associated with greater overall cognitive function.[19]. In rats with Parkinson's disease, sitagliptin improved memory via increasing BDNF expression, which led to an improvement in the animals' overall cognitive function.[20]

Conclusion

The data from the results show increase in social activity with stranger mouse after treatment with sitagliptin for 20 days in social interaction test. Sitagliptin had a strong anti-inflammatory effect by decreasing IL-6 and TNF-α in ASD mice, but this effect is less as compared with risperidone. It is believed that sitagliptin improved behavioral activities and had anti-inflammatory activity by reducing the activation of NF-κB, increasing BDNF expression and rapidly braking down GLP-1R.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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