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Assessing the effects of a DPP IV inhibitor and risperidone on behavioural interactions and lipid peroxidation in a mouse model of induced autism

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ABSTRACT

The autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by early-onset deficits in communication and social interaction. This study investigated the effects of a dipeptidyl peptidase-4 (DPP IV) inhibitor, sitagliptin, in a murine model of ASD induced by prenatal exposure to sodium valproate (VPA). In order to evaluate ASD-like behaviours, an open field test (OFT) was conducted so as to assess locomotor activity and anxiety-related responses. The antioxidant and anti-inflammatory properties of sitagliptin were examined by quantifying levels of reduced glutathione, malondialdehyde (MDA), and interleukin-6. VPA-exposed mice were allocated into four experimental groups: (i) saline control, (ii) VPA + sitagliptin (DPP IV inhibitor), (iii) VPA + risperidone, and (iv) VPA-only. Behavioural assessments *via* OFT were performed on postnatal day 65, followed by biochemical analyses of oxidative stress and inflammatory markers on postnatal day 66. Sitagliptin treatment significantly attenuated oxidative stress and neuroinflammation, leading to marked improvements in aberrant behavioural phenotypes. It exhibited pronounced anxiolytic effects, ameliorating ASD-associated symptoms such as anxiety, hyperactivity, and fearfulness in the OFT. Furthermore, sitagliptin demonstrated superior antioxidant efficacy compared to risperidone, notably in reducing lipid peroxidation. A dose of 10 mg/kg of sitagliptin yielded significant reductions in MDA levels, underscoring its free radical scavenging potential. These findings suggest that sitagliptin may offer a promising therapeutic strategy for mitigating core and associated symptoms of ASD.

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1. Introduction

The autism spectrum disorder (ASD) arises from a complex interplay of environmental and genetic factors during the prenatal and early postnatal periods[1,2]. Prenatal contributors associated with increased ASD incidence include maternal illness and exposure to toxins. Notably, a meta-analysis of over 40,000 ASD cases has identified a correlation between elevated ASD risk and viral infections occurring during labor[3]. A successful ASD therapy is characterized by individualized, integrative strategies that address both cognitive and behavioural dimensions[4]. Currently, risperidone and aripiprazole are the only medications approved by the US Food and Drug Administration for the treatment of ASD-associated symptoms, particularly irritability and aggression[5].

Sitagliptin, a selective inhibitor of the enzyme dipeptidyl peptidase-4 (DPP-4 or DPP IV), exerts its effects through competitive inhibition, resulting in a notable 95% reduction in enzymatic activity over a 12-h period. This inhibition improves glycaemic control by reducing both postprandial and fasting hyperglycaemia, enhancing insulin secretion, and suppressing glucagon levels. As monotherapy, sitagliptin has been shown to reduce glycated haemoglobin levels by approximately 0.8% in patients with type 2 diabetes.

The study of ASD frequently relies on animal models that adopt a multimodal approach encompassing behavioural, neuropathological, physiological, and genetic parameters. While mice carrying mutations in ASD-associated genes are commonly used, comparative studies involving both mice and rats are gaining traction, particularly given rats' more pronounced social behaviours[7,8].

2. Methodology

This study employed 60 healthy adult albino mice weighing between 27 and 40 g. The cohort consisted of 40 females and 20 males, donated by the Ministry of Science and Technology in Baghdad, Iraq. Animals were housed in standard plastic cages at the College of Medicine of the University of Babylon, under controlled conditions: a 12-h light/dark cycle, ambient temperature of 24°C ± 5°C, and relative humidity of 65% ± 5%. Food pellets and water were provided *ad libitum*. All experimental procedures adhered to institutional guidelines for laboratory animal care and were approved by the Department of Pharmacology of the College of Pharmacy of the University of Babylon (approval number: A-0041; date: 5/11/2023).

After a 12-day acclimatization period, pregnant female mice were randomly assigned to two primary groups for comparative analysis. Control groups received intraperitoneal (i.p.) injections of saline. In total, ten groups were formed, each comprising two pregnant females. In order to induce the ASD model, a single i.p. injection of sodium valproate (VPA) at a dose of 600 mg/kg was administered.

On postnatal day 40, offspring were weaned and distributed into eight groups of ten animals each: (i) control groups: group 1 (received oral normal saline), groups 2 and 3 (received oral sitagliptin at doses of 10 mg/kg and 15 mg/kg, respectively), and group 4 (received oral risperidone at a dose of 1 mg/kg), and (ii) VPA-induced groups: group 5 (received oral normal saline), groups 6 and 7 (received oral sitagliptin at doses of 10 mg/kg and 15 mg/kg, respectively), and group 8 (received oral risperidone at a dose of 1 mg/kg).

Hyperactivity was assessed using line crossings and reproductive behaviours in an open field test (OFT). Line crossings, defined as the number of squares traversed along grid lines, served as a measure of locomotor activity. All behaviours were recorded using a Redmi Xiaomi camera (China) for subsequent analysis.

Brain tissue concentrations of interleukin-6 (IL-6), a pro-inflammatory cytokine, were quantified using an enzyme-linked immunosorbent assay (ELISA) kit. Brain tissue levels of malondialdehyde (MDA) were evaluated by the thiobarbituric acid assay method on a spectrophotometer. Finally, reduced glutathione (GSH) levels in the brain tissues were determined according to the Murat Aliskit's colorimetric method. Data were processed and analysed using the SPSS version 26 software. One-way analysis of variance (ANOVA) followed by *post hoc* least significant difference (LSD) testing was employed. Statistical significance was set at $p<0.05$.

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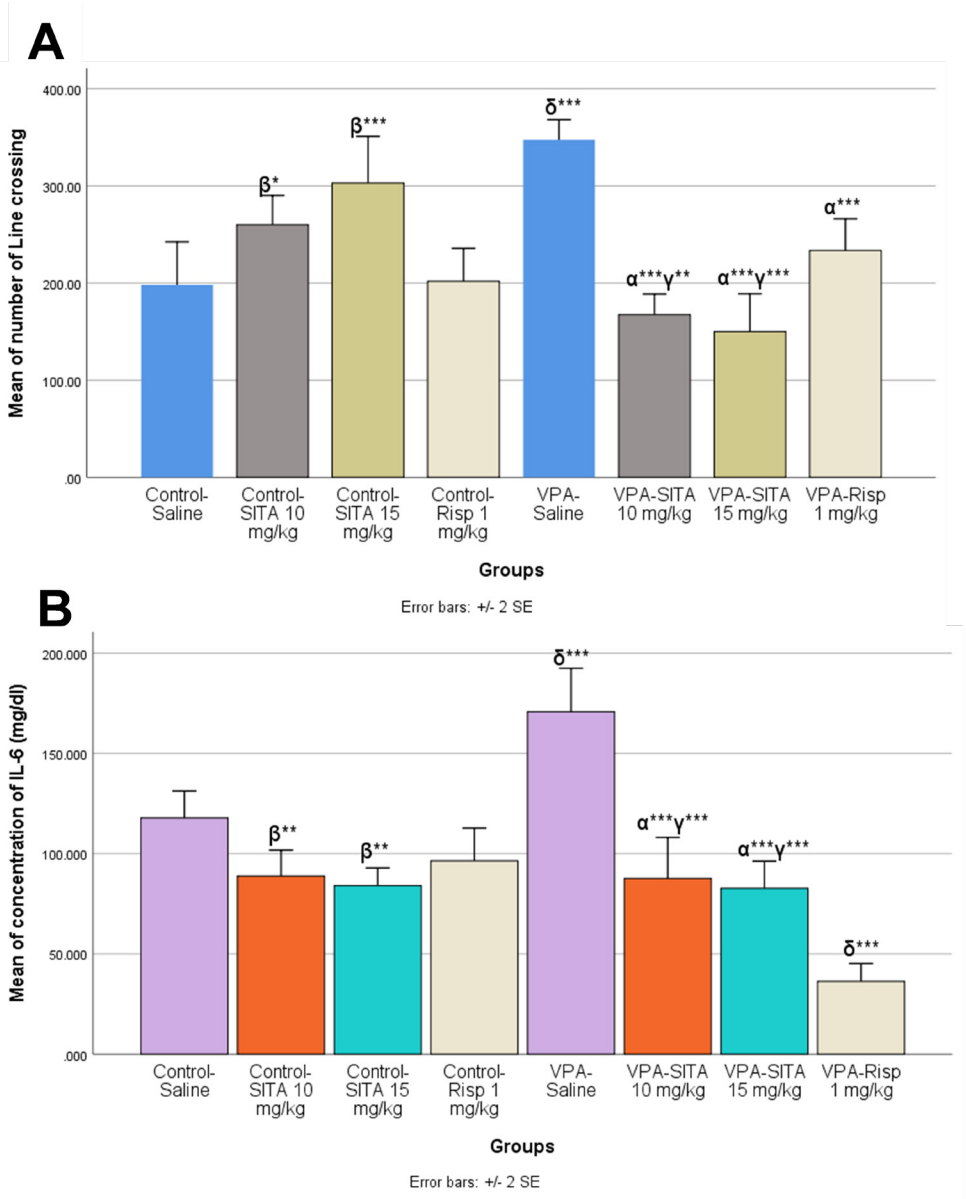

Figure 1. *(A): Effect of sitagliptin (SITA) and risperidone (Risp) on the number of line crossings in the open field test. (B): Effect of SITA and Risp on tissue concentrations of interleukin-6 (IL-6). Notes: *, p<0.05; **, p<0.01; ***, p<0.001; α: versus the sodium valproate (VPA)-saline group; β: versus the control-saline group; γ: versus the VPA-risperidone group. δ: versus other groups.*

3. Results and Discussion

The VPA-saline group exhibited significantly elevated line crossings (p<0.001) compared to all other groups. In contrast, both low- and high-dose VPA-sitagliptin and VPA-risperidone groups demonstrated significantly reduced line crossings (p<0.001) relative to the VPA-saline group. Within the control groups, the high-dose sitagliptin group showed a marked increase in line crossings (p<0.001), while

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the low-dose sitagliptin group also exhibited a significant increase (p=0.014) compared to the control-saline group. Among the VPA-induced groups, the high-dose VPA-sitagliptin group showed a pronounced reduction in line crossings (p<0.001), and the low-dose VPA-sitagliptin group exhibited a highly significant decrease (p=0.009) when compared to the VPA-risperidone group. These findings are illustrated in Figure 1.A.

IL-6 levels were found to be significantly reduced (p<0.001) in both low- and high-dose VPA-sitagliptin and VPA-risperidone groups compared to the VPA-saline group. Furthermore, IL-6 concentrations were significantly lower in the VPA-risperidone group than in either VPA-sitagliptin group (p<0.001). Within the control groups, IL-6 levels were significantly diminished in both the low-dose (p=0.009) and high-dose (p=0.002) sitagliptin groups compared to the control-saline group. These data are presented in Figure 1.B.

The levels of GSH were found to be significantly increased in the 15 mg/kg VPA sitagliptin group as compared with the VPA-risperidone group. Moreover, the levels of MDA were found to be significantly decreased in both dose groups of sitagliptin as compared with the VPA-risperidone group. The dose of 10 mg/kg sitagliptin yielded significant decrease in MDA levels as compared with the dose of 15 mg/kg sitagliptin.

The objective of this study was to establish an ASD model by administering VPA intraperitoneally to pregnant mice on gestational day 12. Offspring exposed to VPA during foetal development exhibited behavioural alterations including reduced social interaction and creativity, increased locomotor activity with diminished exploratory behavior, elevated anxiety scores, and heightened oxidative stress. Treatment with sitagliptin and risperidone mitigated oxidative stress and improved behavioural outcomes in the offspring.

OFT results revealed elevated anxiety in VPA-saline offspring, as evidenced by increased speed, grooming, and rearing behaviours compared to controls. These findings align with previous studies[1,9]. The VPA-sitagliptin group demonstrated a significant reduction in line crossings compared to the VPA-risperidone group. Conversely, the control-sitagliptin group showed a marked increase in line crossings relative to both the control-saline and VPA-risperidone groups. These results corroborate earlier findings indicating that sitagliptin exerts anxiolytic effects in OFT paradigms[10].

This study also evaluated the impact of sitagliptin on IL-6 levels in VPA-exposed mice. Both sitagliptin and risperidone treatments significantly reduced IL-6 concentrations. Notably, IL-6 levels were lower in the VPA-risperidone group than in the VPA-sitagliptin groups. Additionally, IL-6 concentrations were significantly reduced in the 15 mg/kg sitagliptin group compared to the 10 mg/kg group (p<0.001). These findings are consistent with prior research on dementia models demonstrating cytokine modulation by sitagliptin[10].

Reactive oxygen species cause cellular damage (including lipid peroxidation) and interfere with the fluidity of cell membrane. The imbalance between the production and the removing of reactive oxygen species resulted in oxidative stress. Increased levels of GSH and decreased levels of MDA in the VPA-sitagliptin and the VPA-risperidone groups (as compared with VPA-saline group) suggest that both drugs exerted antioxidant activity. Based on these results, sitagliptin proved to have strong antioxidant and free radical scavenging activities. Indirect mechanisms for sitagliptin antioxidant activity upregulated the expression of the nuclear factor erythroid 2-related factor 2[9,10].

4. Conclusion

Our findings indicate that sitagliptin effectively alleviates ASD-like symptoms in mice, demonstrating potent anxiolytic activity in OFT assessments. Sitagliptin also exerted a significant anti-inflammatory effect by reducing IL-6 levels, although its efficacy was lower than that of risperidone. Overall, sitagliptin emerges as a promising candidate for future pharmacological intervention in ASD.

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Conflicts of interest

None exist.

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References

1. Thabault M., Turpin V., Maisterrena A., Jaber M., Egloff M., Galvan L. Cerebellar and striatal implications in autism spectrum disorders: from clinical observations to animal models. *Int. J. Mol. Sci.* 23(4), 2294, 2022. DOI: 10.3390/ijms23042294
2. Loomes R., Hull L., Mandy W.P.L. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. *J. Am. Acad. Child Adolesc. Psychiatry* 56(6), 466–474, 2017. DOI: 10.1016/j.jaac.2017.03.013
3. Jiang H.Y., Xu L.L., Shao L., Xia R.M., Yu Z.H., Ling Z.X., *et al.* Maternal infection during pregnancy and risk of autism spectrum disorders: a systematic review and meta-analysis. *Brain Behav. Immun.* 58, 165–172, 2016. DOI: 10.1016/j.bbi.2016.06.005
4. Hyman S.L., Levy S.E., Myers S.M.; Council on Children with Disabilities, Section on Developmental and Behavioral Pediatrics. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics* 145(1), e20193447, 2020. DOI: 10.1542/peds.2019-3447
5. Masi A., DeMayo M.M., Glozier N., Guastella A.J. An overview of autism spectrum disorder, heterogeneity and treatment options. *Neurosci. Bull.* 33(2), 183–193, 2017. DOI: 10.1007/s12264-017-0100-y
6. Powers A.C., D'Alessio D. Endocrine pancreas and pharmacotherapy of diabetes mellitus and hypoglycemia. In: Brunton L.L., Hilal-Dandan R., Knollmann B.C. (eds). Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 13th Edition. New York, NY: *McGraw-Hill Education*, 863–886, 2018.
7. Satterstrom F.K., Kosmicki J.A., Wang J., Breen M.S., De Rubeis S., An J.Y., *et al.* Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cell* 180(3), 568–584, 2020. DOI: 10.1016/j.cell.2019.12.036
8. Till S.M., Hickson R.D.L., Kind P.C. Cross-species considerations in models of neurodevelopmental disorders. *Trends Neurosci.* 45(3), 171–172, 2022. DOI: 10.1016/j.tins.2021.12.005
9. Lotufo Denucci B., Silva de Lima L., Ferreira Lima Mota I., Rocha Madureira Azevedo J., Germino Veras L., Montenegro Luzardo Bicca J.V., *et al.* Current knowledge, challenges, new perspectives of the study, and treatments of autism spectrum disorder. *Reprod. Toxicol.* 106, 82–93, 2021. DOI: 10.1016/j.reprotox.2021.10.010
10. Khodir S.A., Faried M.A., Abd-Elhafiz H.I., Sweed E.M. Sitagliptin attenuates the cognitive deficits in L-methionine-induced vascular dementia in rats. *Biomed. Res. Int.* 2022, 7222590, 2022. DOI: 10.1155/2022/7222590

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