Basic and Clinical NEUROSCIENCE

May, June 2022 Volume 13, Number 3

Research Paper

Prenatal Zinc Supplementation Ameliorates Hippocampal Astrocytes Activation and Inflammatory Cytokines Expression Induced by Lipopolysaccharide in a Rat Model of Maternal Immune Activation

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Citation Savareh, E., Davoodian, N., Mousaviyan, R., Ghasemi-Kasman, M., Atashabparvar, A., & Eftekhar , E. (2022). Prenatal Zinc Supplementation Ameliorates Hippocampal Astrocytes Activation and Inflammatory Cytokines Expression Induced by Lipopolysaccharide in a Rat Model of Maternal Immune Activation. *Basic and Clinical Neuroscience*, *13*(3), 335-348. http://dx.doi.org/10.32598/bcn.2021.3361.1

http://dx.doi.org/10.32598/bcn.2021.3361.1

Article info:
Received: 27 Apr 2021
First Revision: 15 May 2021
Accepted: 14 Jun 2021
Available Online: 01 May 2022

Keywords:
Maternal immune activation, Maternal zinc supplementation, Schizophrenia, Lipopolysaccharide, Hippocampus, Inflammatory markers

ABSTRACT

Introduction: Evidence suggests that gestational exposure to Lipopolysaccharide (LPS) results in fetal zinc deficiency and eventually neurodevelopmental abnormalities. In this study, we utilized a rat model of Maternal Immune Activation (MIA) to investigate the possible neuroprotective effects of zinc supplementation during pregnancy on hippocampal astrocytes activation as well as inflammatory cytokines expression in adult offspring.

Methods: Pregnant rats received intraperitoneal injections of either LPS (0.5 mg/kg) or saline on Gestational Days (GD) 15 and 16, and orally gavaged with zinc sulfate (30 mg/kg) during pregnancy. Astrocyte density and histological assessment were evaluated in the hippocampus of adult offspring on Postnatal Days (PND) 60 to 62. Also, the mRNA levels of *IL-6, TNF-α, IL-1β, NF-κB*, and *GFAP* were measured using qPCR analysis.

Results: Prenatal exposure to LPS resulted in upregulated expression levels of *IL-6, TNF-α, NF-κB*, and *GFAP* in the hippocampus of adult pups. Moreover, the offspring from the LPS group showed an increased astrocyte density in the CA1 region with no histological alterations in CA1 and CA3 areas. However, maternal zinc supplementation ameliorated the LPS-induced inflammatory alterations.

Conclusion: This study supports the premise that zinc supplementation during pregnancy might be an early treatment option to inhibit hippocampal inflammation induced by the maternal immune response to infectious agents.

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Highlights

- Maternal immune activation induced mild hippocampal inflammation in adult offspring.
- Zinc supplementation suppressed LPS-induced hippocampal inflammation in offspring.
- Zinc might be an early therapeutic option to inhibit neurodevelopmental impairments.

Plain Language Summary

Schizophrenia is a chronic and disabling psychiatric disorder, affecting an estimated one percent of the world's population. To date, the biological mechanisms underlying this mental disorder remain largely elusive, however, research has demonstrated the involvement of both genetic and environmental factors. Of environmental factors, gestational exposure to rubella, influenza, and genital–reproductive infections have gained particular interest among researchers. Based on this evidence, in the present study, we used an animal model of schizophrenia and showed the beneficial effect of zinc supplementation during pregnancy to protect against LPS-induced inflammation in the hippocampus of adult offspring. Collectively, our study provides support for the premise that early treatment might be a suitable option to prevent schizophrenia risk in progeny.

1. Introduction

Schizophrenia is a psychiatric disorder of neurodevelopmental origin. An interaction between genetic and environmental risk factors induces the development of this mental disease. Based on the neurodevelopmental theory, a widely considered theory of schizophrenia, stressful events during specific stages of embryogenesis lead to disturbance in normal brain development with deleterious and long-lasting impact on the brain function and structure later in life (Lang, Puls, Müller, Strutz-Seebohm, & Gallinat, 2007). The gestational infection has gained particular interest among researchers. It is a prenatal risk factor that increases the incidence of schizophrenia in the progeny. Numerous epidemiological and experimental studies support this theory (Boksa, 2010; Khandaker, Zimbron, Lewis, & Jones, 2013; Markham & Koenig, 2011; Meyer, Feldon, & Yee, 2009; Meyer, Yee, & Feldon, 2007). In this regard, maternal immune response to pathogenic agents results in cytokine imbalance in the fetal brain and eventually the manifestation of mental disorders such as schizophrenia (Solek, Farooqi, Verly, Lim, & Ruthazer, 2018). Although the exact mechanism of these detrimental alterations in the brain is unclear, animal models of Maternal Immune Activation (MIA) have been established based on the administration of immunogenic agents such as Lipopolysaccharide (LPS) to pregnant rodents at specific neurodevelopmental phases. Indeed, several studies have supported the face validity of this model to represent several phenotypes related to schizophrenia by detailing the use of the MIA model with various protocols (Alizadeh, Davoodian, Kazemi, Ghasemi-Kasman, & Shaerzadeh, 2020; de Souza et al., 2015; Hao, Hao, Li, & Li, 2010; Mattei et al., 2014; Waterhouse, Roper, Brennan, & Ellenbroek, 2016; Wischhof, Irrsack, Osorio, & Koch, 2015).

Several human studies have provided evidence that the etiology of schizophrenia involves neuroinflammation, abnormal morphology, and functionality of glial cells (Fillman et al., 2013; Müller, 2018; Potvin et al., 2008). Recently, a postmortem study has highlighted a significant upregulation in cellular pathways associated with inflammation in the dorsolateral prefrontal cortex, striatum, and particularly hippocampus of subjects with schizophrenia (Lanz et al., 2019). Consistently, the association between schizophrenia and immune dysregulation has been further supported by numerous MIA animal model studies, which dominantly evaluated the density or activity of microglia and astrocytes in different brain regions (Solek et al., 2018). Although microglia are the principal innate immunity component in the brain, astrocytes, the most numerous cell in the Central Nervous System (CNS), are also crucial in the brain's immune activity (Farina, Aloisi, & Meinl, 2007). As such, remarkable astrogliosis, in addition to neuroinflammation, has been detected in the hippocampus of pups with prenatal exposure to *LPS* or *IL-6* (Hao et al., 2010; Samuelsson, Jennische, Hansson, & Holmang, 2006). This finding is further supported by a recent study demonstrating the significant astrogliosis in both the prefrontal cortex and hippocampus of adult pups born

to dams treated with polyriboinosinic–polyribocytidylic acid (poly[I:C]) (Ding et al., 2019). These results indicate that maternal infection leads to long-lasting alterations in the offspring's brain. However, there is a controversy surrounding the changes in the density and activity of astrocytes in both postmortem brains of schizophrenic patients (Trepanier, Hopperton, Mizrahi, Mechawar, & Bazinet, 2016) and MIA animal models (de Souza et al., 2015). This controversy might be because of different experimental designs.

Epidemiological studies have documented that the response to MIA, and not a specific kind of pathogen, is involved in the etiology of neurodevelopmental disorders, including schizophrenia (Brown et al., 2004; Solek et al., 2018; Yolken, Dickerson, & Fuller Torrey, 2009). The obvious main response to maternal immune challenge is the increased production of pro-inflammatory cytokines; however, the secondary consequences of systemic immune activation also need to be considered (Reisinger et al., 2015). Accordingly, gestational exposure to LPS acts as a potent inducer of maternal Metallothionein (MT), resulting in maternal hypozincemia and, ultimately, fetal zinc deficiency (Carey, Berbée, Coyle, Philcox, & Rofe, 2003; Coyle, Tran, Fung, Summers, & Rofe, 2009). Zinc is an essential and abundant trace element required for the proper function of numerous proteins. Also, It greatly influences a broad spectrum of cellular processes, including immunity, wound healing, and normal brain functions (Chasapis, Loutsidou, Spiliopoulou, & Stefanidou, 2012). Zinc dyshomeostasis contributes to a range of psychiatric diseases, such as depression and schizophrenia (Portbury & Adlard, 2017). This issue is further supported by recent human studies demonstrating a significant reduction in the serum concentration of zinc in schizophrenic subjects (Cai et al., 2015; Cao et al., 2019). In addition, zinc supplementation during pregnancy is confirmed to reduce neurobehavioral alterations and teratogenicity, as well as fetal death induced by LPS in MIA animal model (Alizadeh et al., 2020; Carey et al., 2003; Coyle et al., 2009; Kirsten et al., 2015). However, the cellular mechanism underlying the protective effect of zinc supplementation against LPS-induced impairments is yet to be fully clarified.

Because of the putative role of the hippocampus in neurogenesis and learning, as well as its involvement in neuropsychological impairments associated with schizophrenia (Ewing & Winter, 2013), in this study, we utilized the MIA animal model to evaluate the possible neuroprotective effect of zinc supplementation during pregnancy on astrocyte activation and several inflammatory mediators in the hippocampus of adult pups.

2. Materials and Methods

Study animal

Female and male Wistar rats (female: 200-230 g; male: 250-300 g) were obtained from the animal house of Hormozgan University of Medical Sciences. Animals were kept under standard environmental conditions (temperature: 22ºC, humidity: 60%-70%, 12 h light-dark cycle), with unlimited access to food and tap water. In the present study, we used 8-week-old male offspring selected based on the results of our previous study (Alizadeh et al., 2020). All experimental procedures were based on the National Institutes of Health guide for the care and use of laboratory animals and approved by the Ethics Committee of Hormozgan University of Medical Sciences (HUMS) (IR.HUMS.REC.1397.276). To minimize the number and the suffering of animals based on the Three Rs principle, we shared the treated animals in two other published articles (Alizadeh et al., 2020; Mousaviyan et al., 2021).

Study treatment

Adult male and female rats were housed overnight, and the first day of pregnancy was confirmed by the presence of spermatozoa in vaginal smears, which was designated as gestational day 1 (GD1). Pregnant dams were randomly assigned into four treatment conditions with 6 litters per group: control, pregnant dams received Intraperitoneal (IP) injections of saline at GD 15 and 16; LPS, pregnant dams received LPS injections (0.5 mg/kg, IP, Escherichia coli L2630) (Waterhouse et al., 2016; Wischhof et al., 2015) at GD15 and 16; LPS+Zinc, pregnant dams received LPS injections (0.5 mg/kg, IP) at GD15 and 16 and orally gavaged with zinc sulfate (30 mg/kg) (Moazedi, Ghotbeddin, & Parham, 2007) during pregnancy; and Zinc, pregnant dams received IP injections of saline at GD15 and 16 and orally gavaged with zinc sulfate (30 mg/kg) during pregnancy. The control and LPS groups were administered with an equal volume of water during pregnancy by gavage. The experimental timeline is provided in Figure 1. Based on sex and treatment, the resulting offspring were weaned on postnatal day 21 (PND21) and maintained undisturbed until PND60. For sample size calculation, we conducted the resource equation method (Charan & Kantharia, 2013). To minimize the litter effects, one male offspring from each litter was randomly selected for future analysis. The remaining pups were used for other experiments that are not reported here.

Tissue collection, RNA isolation, and qPCR analysis

At PND 60, six male pups from each group (n=6, one pup per litter and 6 litters per group) were sacrificed using Carbon Dioxide (CO_2) euthanasia. The whole brain was rapidly removed, and placed on ice, followed by the microdissection of the hippocampus (Chiu, Lau, Lau, So, & Chang, 2007). The tissues were immediately snap-frozen and kept at -80°C.

The following experimental procedures were carried out based on MIQE guidelines (Bustin et al., 2009). Total RNA was extracted using TRIzol™ Reagent (Sigma-Aldrich, USA), based on the manufacturer's instructions. After assessing the RNA integrity using agarose gel electrophoresis, RNA (1 µg) of each sample was reverse-transcribed using the cDNA synthesis kit (Thermo Ficher, USA), as described by the manufacturer's protocol. qPCR reactions were performed using a Mic qPCR system (Australia) with the primer sets for *IL-6, TNF-α,* Nuclear Factor Kappa B (*NF-κB*), *GFAP,* and *IL-1 β* as the target genes and GAPDH as a reference gene (Table 1). The reactions were conducted in SYBR Premix Ex Taq II (Takara, Japan) with a three-step protocol, as described previously (Alizadeh et al., 2020). After normalization with GAPDH, relative gene expression analysis was calculated using the $2^{-\Delta\Delta Ct}$ method.

Immunostaining

Immunofluorescence staining was carried out, as described previously (Mousaviyan et al., 2021). After anesthetization with ketamine/xylazine (100 mg/kg - 10 mg/kg), animals (n=4/group) were transcardially perfused with Phosphate-Buffered Saline (PBS) and 4% paraformaldehyde (PFA). The brain tissues were harvested, post-fixed in PFA overnight, and finally immersed in sucrose solution (30%) for 48 h. After freezing in the O.C.T compound, coronal sections of the hippocampus (6 µm) were obtained by a cryostat apparatus (MICROM HM 525, Germany) and mounted on charged slides.

For immunostaining, tissue sections were washed with PBS for three 5-min, followed by blocking in 10% normal goat serum and 0.3% Triton X-100 for 1h. The slides were then incubated overnight with rabbit Anti-Glial Fibrillary Acidic Protein (GFAP) (1:400, Z0334, Dako) at 4˚C. Afterward, the slides were washed with PBS for three 10-min periods and incubated with Goat anti-rabbit Alexa Fluor®594 (1:1000 dilution, ab150080) secondary antibody for 1 h, followed by staining with 4',6-diamidino-2-phenylindole (DAPI) for 10 min. Subsequently, the images were obtained by a fluorescence microscope (Nikon, Japan). The fluorescence images were manually quantified as the number of GFAP positive cells soma/total cells using ImageJ 1.42 software (NIH, USA).

Histopathological examination

Similar to the immunofluorescence staining procedure, animals (n=4/group) were perfused with PBS and 4% PFA. Then, the cerebral samples were rapidly harvested and stored in 4% PFA overnight. After dehydration with a series of alcohol and paraffin embedding, the tissues were cut into 6-µm thick coronal sections. Finally, H&E staining was performed, and the slides were evaluated under a microscope.

Statistical analysis

Data analysis was conducted by GraphPad Prism 6 (GraphPad Software Inc. San Diego, CA, USA). The data from qPCR and immunostaining were analyzed by a 2-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Prenatal treatment (saline vs LPS) and maternal supplementation (vehicle vs zinc) was considered between-group variables. The data are presented as Mean±SD, and $P \leq 0.05$ was considered significant.

3. Results

Prenatal zinc supplementation suppressed the up-regulation of pro-inflammatory markers induced by LPS

To evaluate the LPS-induced inflammatory reaction in the hippocampus of the offspring and the possible protective effect of prenatal zinc supplementation, we measured the expression levels of *IL-6, TNF-α, NF-κB, GFAP*, and *IL-1 β* by the qPCR technique. As illustrated in Figure 2a, the expression level of *IL-6* was differentially affected by LPS, with a moderate increase in the hippocampus of the offspring prenatally exposed to LPS compared to the corresponding control (the main effect of prenatal treatment, $F_{1, 20}=8.829$, $P=0.0075$; LPS group vs control group Tukey's multiple comparison, $P<0.01$), and this effect was prevented by zinc supplementation in the LPS+Zinc group (interaction of main effects, $F_{1, 20}=9.403$, $P=0.0061$; LPS+Zinc group vs. LPS group Tukey's multiple comparison, $P<0.05$) (Figure 2a). Furthermore, a significant effect of prenatal treatment was detected for tumor necrosis factor-alpha *(TNF-α)* mRNA level, reflected by a slight increase in the hippocampus of LPS-exposed pups related to the control group (main effect of prenatal treatment, $F_{1, 16}=8.577$, $P=0.0083$; LPS group vs. control group

Table 1. Primer sets applied for qPCR

Gene	Accession Number	Sequences
IL-6	NM_012589.2	5′-TGATGGATGCTTCCAAACTG-3′ 5′-GAGCATTGGAAGTTGGGGTA-3′
IL-1B	NM_031512.2	5′-GCTGTGGCAGCTACCTATGTCTTG-3′ 5′-AGGTCGTCATCATCCCACGAG-3′
TNF-A	NM_012675.3	5′-AAATGGGCTCCCTCTCATCAGTTC-3′ 5′-TCTGCTTGGTGGTTTGCTACGAC-3′
nf-κB	XM_006233360.3	5′-TGCAGAAAGAAGACATTGAGGTG-3′ 5′-AGGCTAGGGTCAGCGTATGG-3′
GFAP	NM_017009.2	5′-TGGCCACCAGTAACATGCAA-3′ 5′-CAGTTGGCGGCGATAGTCAT-3′
GAPDH	XM_017593963.1	5′-ACGGCAAGTTCAACGGCACAG-3′ 5′-GACATACTCAGCACCAGCATCACC-3′

Tukey's multiple comparison, P<0.01), that returned to the control level upon maternal zinc supplementation in the LPS+Zinc group (interaction of main effects, $F_{1, 16}$=8.401, P=0.0089; main effect of maternal supplementation, $F_{1, 16}$=4.754, P=0.0413; LPS+Zinc group vs.

LPS group Tukey's multiple comparison, P<0.01) (Figure 2c). However, gene expression analysis revealed no significant effects of prenatal treatment ($F_{1, 17}$=0.7645, P=0.3923), maternal supplementation ($F_{1, 17}$=0.1752, P=0.6800), and their interaction ($F_{1, 17}$=1.507, P=0.2339) for *IL-1β* in the experimental groups (Figure 2b). Conversely, *LPS* treatment had a moderate but significant effect on the expression level of *NF-κB*, depending on the prenatal supplementation. *NF-κB* expression level was significantly upregulated in the offspring from LPS-treated mothers compared to the control pups (main effect of the prenatal treatment, $F_{1, 17}$=6.305, P=0.0207; LPS group vs. control group Tukey's multiple comparison, P<0.01), whereas this increase was suppressed in the LPS+Zinc group (interaction of main effects, $F_{1, 17}$=14.12, P=0.0012; main effect of the maternal supplementation, $F_{1, 17}$=5.549, P=0.0288; LPS+Zinc group vs. LPS group Tukey's multiple comparison, P<0.01) (Figure 2d). Similarly, there was a significant increase in the GFAP mRNA level in the pups of LPS-exposed mothers related to the control group (main effect of the prenatal treatment, $F_{1, 20}$=4.822, P=0.0401; LPS group vs. control group Tukey's multiple comparison, P<0.05), while in the offspring of the LPS+Zinc group, GFAP expression level was approximately back to the control level (LPS+Zinc group vs. LPS group Tukey's multiple comparison, P<0.05). However, no significant interaction or main effect of the maternal supplementation were found for GFAP expression level (interaction of main effects, $F_{1, 20}$=4.157, P=0.0549;

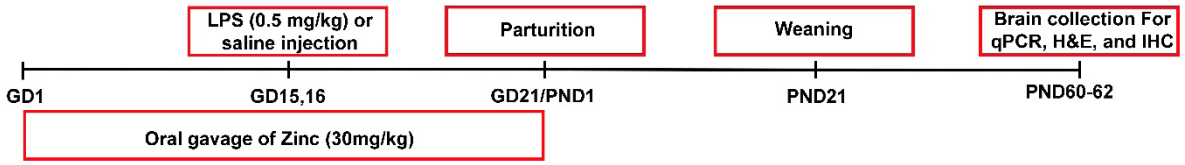

Figure 1. A schematic diagram describing the experimental timeline

On Gestation Days (GD) 15 and 16, pregnant dams were intraperitoneally administered with either LPS (500 µg/kg) or saline and gavaged with zinc sulfate (30 mg/kg)/vehicle. The resulting offspring were submitted to qPCR, immunostaining, and morphological analysis at PND60.

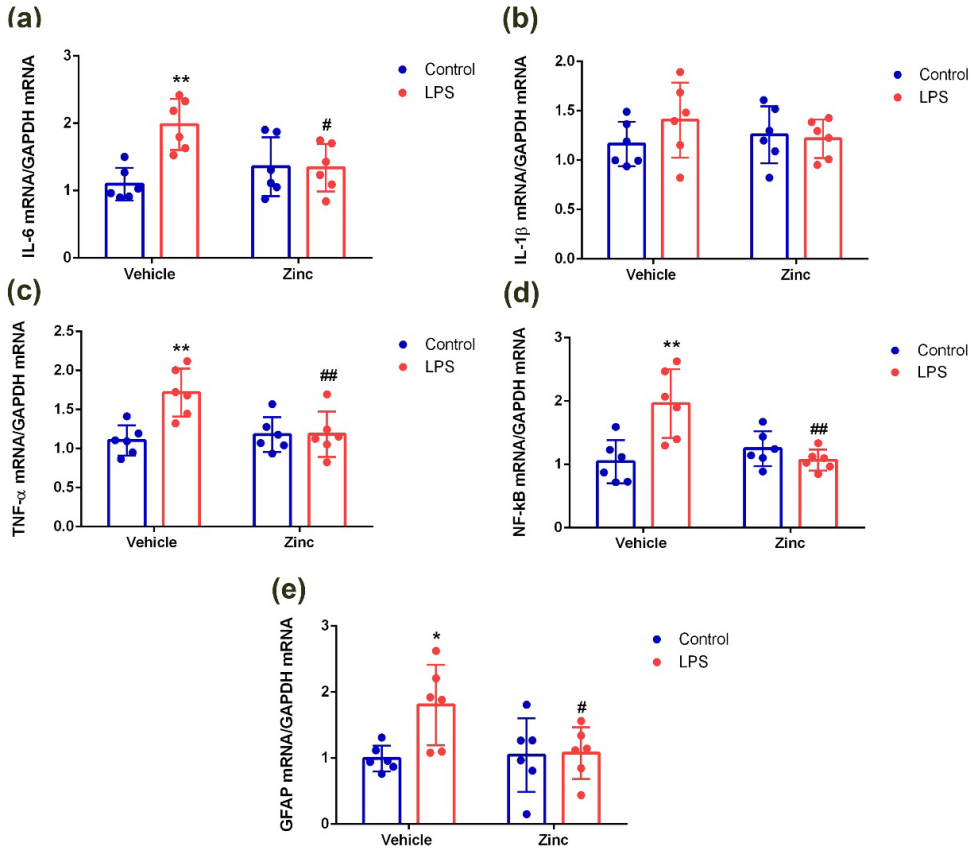

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Figure 2. Prenatal LPS treatment enhanced the expression of pro-inflammatory mediators in the hippocampus of offspring at PND60, suppressed by maternal zinc supplementation

Prenatal LPS exposure significantly induced the expression of *IL-6* (a), *TNF-a* (c), *NF-κB* (d), and *GFAP* (e) in the hippocampus of pups, which was mitigated by maternal zinc supplementation. The data are presented as mean±SD, n=6 per group.

*P<0.05, **P<0.01, and ***P<0.001 compared to the control group. #P<0.05, ##P<0.01, and ###P<0.001 compared to the LPS group.

main effect of maternal supplementation, $F_{1, 20}$=3.139, P=0.0917) (Figure 2e).

Prenatal zinc supplementation alleviation effect on the increased density of astrocytes induced by LPS in the CA1 hippocampus

To examine whether the alterations in the mRNA levels of GFAP and pro-inflammatory markers were associated with the changes in astrocyte density, we carried out immunostaining in CA1 and CA3 areas (Figures 3 and 4). In the CA1 region of the hippocampus, a significant effect of prenatal treatment was revealed (main effect of prenatal treatment, $F_{1, 12}$=13.86, P=0.0029), with pups born to LPS-exposed dams exhibiting a marked increase in GFAP$^+$cells compared to the control group (LPS group vs. control group Tukey's multiple comparison, P<0.01). This effect was restrained by the maternal zinc supplementation in the LPS+Zinc group (interaction of main effects, $F_{1, 12}$=11.32, P=0.0056; LPS+Zinc group vs LPS group Tukey's multiple comparison, P<0.05), with no main effect of the maternal supplementation (main effect of maternal supplementation, $F_{1, 12}$=3.382, P=0.0908) (Figure 3b). However, in CA3 area of the hippocampus, no statistically significant effect for prenatal treatment, maternal supplementation, as well as their interaction was detected on the astrocyte density (interaction of main effects, $F_{1, 12}$=0.0, P > 0.9999; main effect of prenatal treatment, $F_{1, 12}$=0.2297, P=0.6404; main effect of maternal supplementation) $F_{1, 1}$ =0.2297, P=0.6404) (Figure 4b).

No histological changes were observed in CA1 and CA3 hippocampus of offspring

H&E staining was performed to examine the possible histological alterations, including changes in the arrangement and morphology structure of cells in both CA1 and CA3 hippocampus of offspring. As depicted in Figure 5a, the CA1 area of the hippocampus from all groups

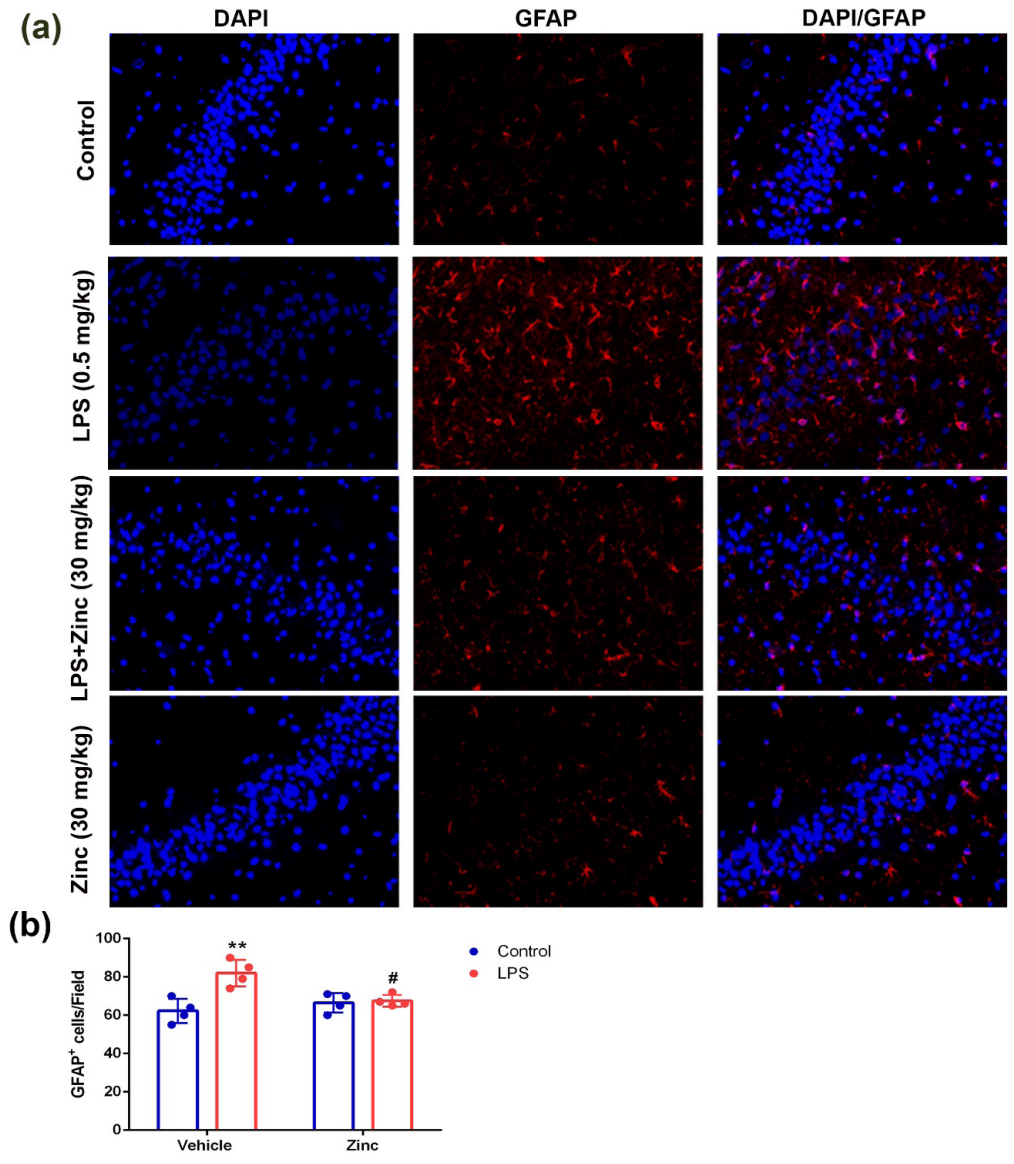

Figure 3. Immunostaining for GFAP in the CA1 hippocampus of MIA offspring

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Immunofluorescence images (a) and the quantified graph (b) of *GFAP* in the CA1 area of pups at PND60. The data are presented as Mean±SD, n=4 per group. Scale bar: 50 µm, **P<0.01 compared to the control group and #P<0.05 compared to the LPS group.

exhibited arranged neurons with normally rounded nuclei (Figure 5a). Similarly, we detected no evidence of histological changes in the CA3 hippocampus of pups in all experimental groups (Figure 5b).

4. Discussion

Maternal infection is considered a prenatal risk factor associated with deleterious effects on the brain function and structure in the progeny (Garay, Hsiao, Patterson, & McAllister, 2013). In our previous study, we demonstrated that prenatal exposure to LPS on GD15 and 16 results in significant behavioral impairments in adult offspring as a phenotype associated with schizophrenia (Alizadeh et al., 2020). Remarkably, these behavioral deficits were observed only among male pups, which were selected for further investigation. In this study, our findings revealed the long-lasting alterations in the hippocampus of adult offspring prenatally exposed to LPS, characterized by the enhanced expression levels of *IL-6, TNF-α, NF-κB*, and *GFAP* as well as increased astrocyte density in the CA1 region of the hippocampus. Furthermore, the mentioned changes were alleviated by maternal zinc supplementation.

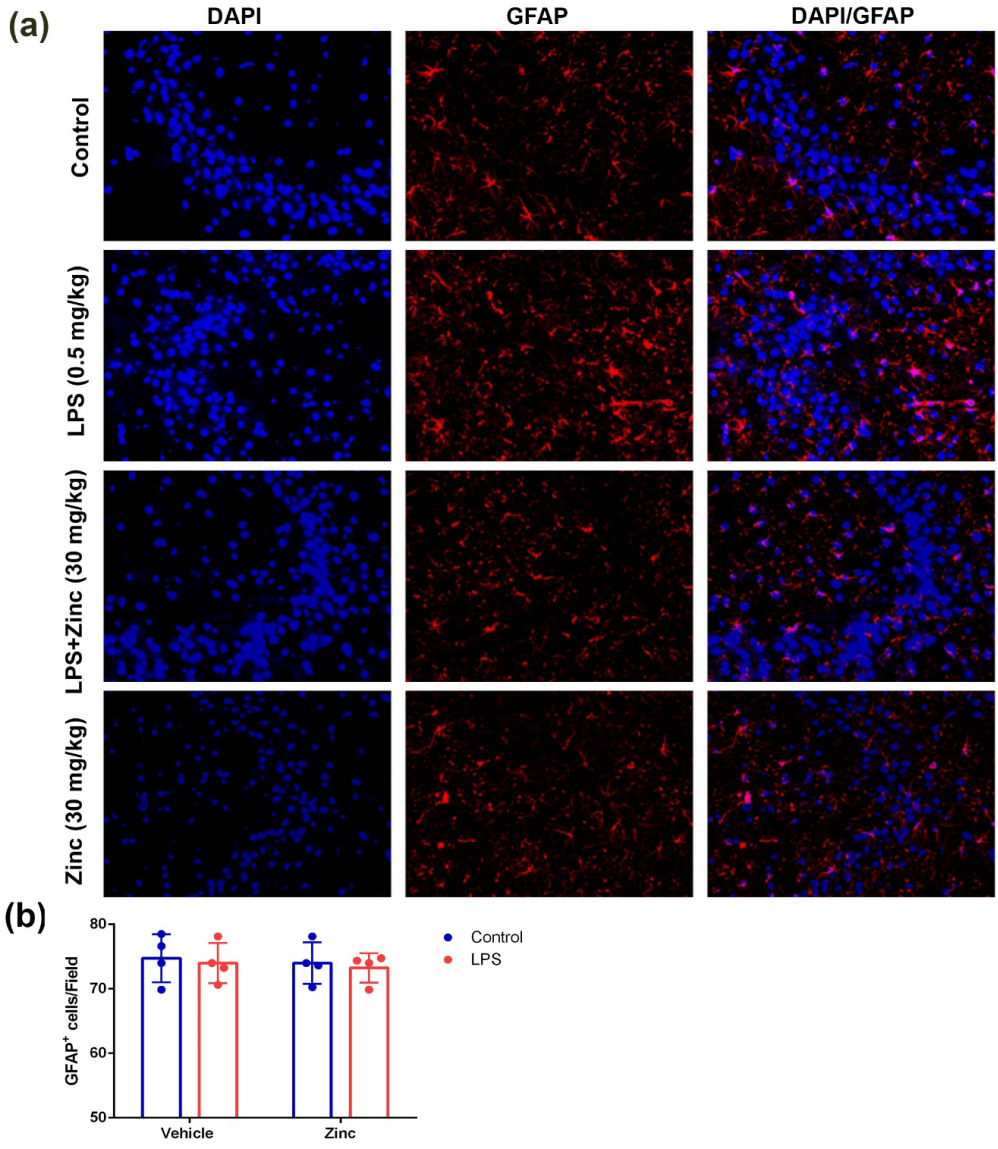

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Figure 4. Immunostaining for GFAP in the CA3 hppocampus of MIA offspring

Immunofluorescence images (a) and the quantified graph (b) of *GFAP* in the CA3 area of pups at PND60. The data are presented as Mean±SD, n=4 per group. Scale bar: 50 µm.

Hippocampus is a region in the brain with a prominent role in memory, learning, and neurogenesis. Accordingly, both human and animal studies have repeatedly highlighted the importance of the hippocampus as one of the main areas involved in the pathophysiology of schizophrenia (Antoniades et al., 2018; de Souza et al., 2015; Hao et al., 2010; Lieberman et al., 2018). This issue is further evidenced by the well-described reduction in hippocampal volume in schizophrenic patients and MIA offspring (Adriano, Caltagirone, & Spalletta, 2012; van Erp et al., 2016; Zhou, 2015). Furthermore, a recent postmortem study has detected a robust upregulation of inflammatory pathways, especially the *IL-6* pathway, in the hippocampus of subjects with schizophrenia. This finding supports the persistent neuroinflammation in the brain of schizophrenic patients (Lanz et al., 2019). However, in MIA model studies, contradictory findings have been reported regarding the alterations in hippocampal pro-inflammatory markers of resulting offspring. Herein, we detected the increased mRNA levels of *NF-κB*, *TNF-α*, and *IL-6* with an unchanged expression level of *IL-1β* in the hippocampus of the offspring born to LPS-treated mothers. In this regard, Pups exposed in utero to poly I:C (4 mg/kg, IV) exhibited the upregulation in the expression of both *TNF-α* and *IL-1β* in the hippocampus at PND120 (Mattei et al., 2014). In the same region,

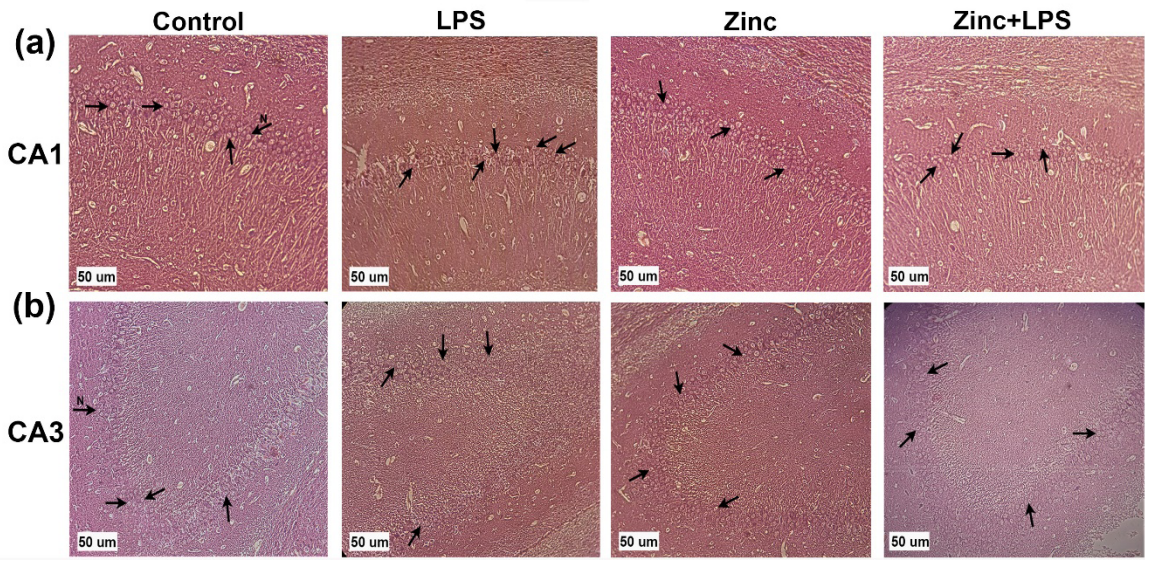

Figure 5. Histological assessment of CA1 and CA3 hippocampus

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No histological alterations were observed in both CA1 (a) and CA3 (b) areas for the offspring in all experimental groups. Black arrows represent the normal (N) neurocyte. Scale bar: 50µm, (n=4).

another study showed the elevated mRNA level of *IL-6* in adult offspring prenatally exposed to *IL-6* (9 µg/kg, IP) (Samuelsson et al., 2006). However, protein levels of *IL-6, IL-1β,* and *TNF-α* were reported unchanged in the hippocampus of pups prenatally exposed to poly I:C (5 mg/kg, IV) (Giovanoli et al., 2015). Similar results were demonstrated by a recent study using a different dose of poly I:C (10 mg/kg, IV) (Ding et al., 2019). In addition, in the only MIA study, according to our knowledge, that examined the expression levels of different inflammatory genes, the mRNA level of *NF-κB* was found to be unchanged in the hippocampus of offspring at PND21 (Zhou, 2015). Methodological differences, including the difference in dose, immunogenic substances, and the gestational stage during which the MIA is induced, might be the reasons behind the discrepancies in the reported results. Notably, our study revealed the upregulation in GFAP expression level and the increased GFAP positive cells in the CA1 hippocampus of offspring prenatally exposed to LPS with no alteration in the CA3 region. This discrepancy observed in hippocampal CA1 and CA3 is probably because of the difference in astrocyte sensitivity and vulnerability of these regions, which is reported to be responsible for the selective neural death in CA1 after forebrain ischemia (Sun, Fukushi, Wang, & Yamamoto, 2018). Using a similar protocol, de Souza et al. noted the increased level of GFAP protein (measured by ELISA) and GFAP immunocontent in the CA1 hippocampus of adult rats born to LPS-challenged mothers (0.5 mg/kg, IP) (de Souza et al., 2015). Consistently, adult offspring of dams treated with LPS (0.79 mg/kg, IP) exhibited higher numbers of GFAP immunoreactive cells related to the control (Hao et al., 2010). In contrast, mice exposed prenatally to poly I:C (5 mg/kg, IV) showed no alterations in the density of GFAP-positive astrocytes in CA1, CA3, and dentate gyrus areas at PND28 and 140 (Giovanoli et al., 2015). Different infectious agents and their concentrations might explain these conflicting results. Considerably, human studies have also highlighted the pivotal role of astrocytes in the etiology and pathophysiology of schizophrenia (Catts, Wong, Fillman, Fung, & Shannon Weickert, 2014; Tarasov et al., 2020). Therefore, dysregulation of astrocytes function, which are morphologically and functionally associated with neurons, might be a new point of view to illustrate metabolic and transmitter alterations in the brain of schizophrenic patients (Tarasov et al., 2020). Moreover, we performed histological evaluation in the CA1 and CA2 areas of hippocampus of adult offspring. In contrast to the previous study in which a disordered and reduced number of nuclei were reported in the CA1 hippocampus of pups born to LPS-challenged mothers (0.79 mg/kg, IP) (Hao et al., 2010), we detected no significant histological changes in both areas. Similarly, the number of neurons is unchanged in the hippocampus of schizophrenic patients (Heckers & Konradi, 2002), as well as in the prenatally immune-challenged offspring in the schizophrenic MIA model (de Souza et al., 2015). This finding supports the hypothesis that disruption in neural connectivity (Ruiz, Birbaumer, & Sitaram, 2013), instead of reduced neuron number, might be involved in the development of this mental disease.

As a complex mental health disease, schizophrenia is characterized by three major manifestations: positive, negative, and cognitive symptoms. Currently, the available pharmacological treatment options mainly provide relief for positive symptoms with no effective treatment for negative and cognitive symptoms (Patel, Cherian, Gohil, & Atkinson, 2014). With this background, the discovery of satisfactory pharmacological options and early therapeutic interventions is of interest. Using an MIA animal model with well-established face and predictive validity (Reisinger et al., 2015), we found that maternal zinc supplementation alleviated LPS-induced increased expression of *IL-6, TNF-α, NF-κB, GFAP*, and also *GFAP* positive cells in the hippocampus of male pups. It has been reported that gestational infection with LPS is correlated with maternal and fetal hypozincemia mainly because of the stimulation of metallothionein synthesis in the maternal liver (Carey et al., 2003; Coyle et al., 2009). In addition to being involved in innate and adaptive immunity, zinc is strongly required for normal brain functions. Meanwhile, fetal hypozincemia can eventually lead to neurodevelopmental damage in the offspring (Chua, Cowley, Manavis, Rofe, & Coyle, 2012; Coyle et al., 2009). In support of this possibility, findings of several studies demonstrate the beneficial effect of zinc supplementation during pregnancy to protect against fetal death, neurobehavioral impairments, autistic-like behaviors, and preterm delivery induced by LPS with unclear molecular mechanisms (Alizadeh et al., 2020; Chen et al., 2012; Chua et al., 2012; Kirsten et al., 2015). One possible mechanism is that zinc supplementation during pregnancy might counteract the LPS-induced reduction of zinc availability to the fetus and ultimately prevent the abnormal neurodevelopment in the progeny. Another mechanism can be explained by the antioxidant and anti-inflammatory properties of this element. In support of this, numerous studies have consistently documented the link between zinc deficiency and increased production of oxidative stress and inflammatory markers (Jarosz, Olbert, Wyszogrodzka, Młyniec, & Librowski, 2017). The literature demonstrates the negative regulatory effect of zinc on *NF-κB* as one of the main inflammatory pathways (Jarosz et al., 2017). This signaling cascade positively regulates the expression of pro-inflammatory genes *IL-6, TNF-α, IL-1β*, and so on (Lawrence, 2009). In this context, it has been suggested that zinc exerts an inhibitory effect on LPS-induced *NF-κB* and eventually suppresses the expression of inflammatory mediators, which further supports our findings (Jarosz et al., 2017). As mentioned earlier, we also found that maternal zinc supplementation alleviated LPS-induced increment in GFAP mRNA level and astrocyte density in the CA1 hippocampus of pups. To our knowledge, there is no MIA model study evaluating the effect of maternal zinc supplementation on the astrocyte density of adult offspring. However, in the fetal hippocampus at GD18, the previous study reported the protective influence of prenatal zinc treatment on LPS-induced astrogliosis (Chua et al., 2012).

Finally, this study has the following limitations. MIA animal model is profoundly considered a suitable tool to evaluate pathomechanism and develop new therapeutic agents for some of the most complex mental diseases, including schizophrenia (Reisinger et al., 2015). However, there is a stigma associated with this model, as this approach cannot represent all behavioral and pathological features of a particular neurodevelopmental disease. Additionally, the main focus of this study was to investigate hippocampal astrocyte changes in adult offspring. It should be taken into consideration that other glial cells, including microglia cells, have a role in producing inflammatory markers. Despite the abundant data for the cytokine disturbance in the brain of MIA offspring, literature has provided conflicting results about microglial activation in different brain regions. While some studies have documented an increase in microglial activation, others have demonstrated no significant alterations (Bergdolt & Dunaevsky, 2019; Solek et al., 2018). Therefore, the investigation of possible changes in astrocyte and microglia density in different hippocampal regions of MIA offspring is of utmost importance.

5. Conclusion

In conclusion, in the present study, we utilized the MIA animal model to examine the beneficial effect of maternal zinc supplementation in protection against LPS-induced hippocampal inflammation in adult offspring. Our findings showed that prenatal LPS exposure induced long-lasting alterations in the hippocampus of the resulting offspring, evidenced by the increased expression of *NF-κB, TNF-α, IL-6, GFAP*, and the astrocyte density in the CA1 area. In addition, our findings demonstrated that zinc supplementation during pregnancy mitigated the mentioned LPS-induced impairments. Therefore, considering a lack of comprehensive therapeutic strategy for schizophrenia, zinc supplementation during pregnancy might be an early treatment option to inhibit neurodevelopmental abnormalities induced by the maternal immune response to infectious agents.

The analyzed data are available from the corresponding author upon reasonable request.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures were based on the National Institutes of Health guide for the care and use of laboratory animals. The Ethics Committee of Hormozgan University of Medical Sciences (HUMS) approved tthe research (IR.HUMS.REC.1397.276).

Funding

This study was supported by the Research Vice-chancellor of Hormozgan University of Medical Sciences (HUMS) (Grant No.: 970280).

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to acknowledge the technical advice provided by Haniyeh Kazemi.

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