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Prenatal zinc prevents communication impairments and BDNF disturbance in a rat model of autism induced by prenatal lipopolysaccharide exposure

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ABSTRACT

Aims: Previous investigations by our group have shown that prenatal exposure to lipopolysaccharide (LPS), which mimics infections by Gram-negative bacteria, induced autistic-like behavior. No effective treatment yet exists for autism. Therefore, we used our rat model to test a possible treatment for autism. We selected zinc as the prenatal treatment to prevent or ease the impairments induced by LPS because LPS induces hypozincaemia.
Materials and methods: We evaluated the effects of LPS and zinc on female reproductive performance. Communication, which is impaired in autism, was tested in pups by ultrasonic vocalizations. Plasma levels of brain-derived neurotrophic factor (BDNF) were determined because it has been considered an autism important biomarker.
Key findings: Prenatal LPS exposure reduced offspring number and treatment with zinc prevented this reduction. Moreover, pups that were prenatally exposed to LPS spent longer periods without calling their mothers, and posttreatment with zinc prevented this impairment induced by LPS to the same levels as controls. Prenatal LPS also increased BDNF levels in adult offspring, and posttreatment with zinc reduced the elevation of BDNF to the same levels as controls.
Significance: BDNF hyperactivity was also found in several studies of autistic patients. Together with our previous studies, our model of prenatal LPS induced autistic-like behavioral, brain, and immune disturbances. This suggests that it is a valid rat model of autism. Prenatal zinc prevented reproductive, communication, and BDNF impairments. The present study revealed a potential beneficial effect of prenatal zinc administration for the prevention of autism with regard to the BDNF pathway.

1. Introduction

Autism is a developmental brain disorder with a higher prevalence in males and characterized by social deficits, communication abnormalities, repetitive behaviors, and sometimes changes in some blood and brain biomarkers, such as brain-derived neurotrophic factor (BDNF) [1,2]. One in every 100 children is diagnosed with autism [3,4]. The risk factors seem to include genetic and perinatal environmental agents, such as gestational zinc deficit, although its exact etiology remains unknown [5–7].

Previous investigations by our group have shown that prenatal treatment with lipopolysaccharide (LPS; 100 μg/kg, intraperitoneally [i.p.]), an endotoxin that mimics infection with Gram-negative bacteria, in rats on gestational day (GD) 9.5 impaired communication and socialization and induced repetitive/restricted behavior in male rats. However, the behavior of female rats was not altered [8,9]. These results suggest that our model of prenatal LPS exposure induces autism-like effects in offspring [9]. Moreover, we observed an increase in serum interleukin-1β (IL-1β) levels in adult offspring [10], a finding already reported in several autistic patients [11–13]. The effects of maternal LPS exposure on the developing fetal brain have been suggested to be mediated by the induction of proinflammatory cytokines within the maternal circulation and placenta [14–16].

No effective treatment yet exists for autism, with no consensus on the type of medication to prescribe [17]. A few drugs have been approved by the U.S. Food and Drug Administration, but they have limited efficacy, treat only part of the symptoms, and trigger adverse effects [18]. Therefore, the purpose of the present study was to use our rat model of autism to test a treatment for autism. We selected zinc as the prenatal treatment to prevent or ease the impairments induced by LPS. Cytokines produced after LPS exposure induce metallothionein, which sequestrates zinc and induces maternal and fetal hypozincaemia [19]. Coyle's group reported that hypozincaemia induced by LPS leads to teratogenesis and that zinc supplementation prevented some reproductive and behavioral impairments [19,20]. Human studies have

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investigated nutritional supplementation with zinc for autism treatment [21,22]. According to Theoharides and colleagues [7], however, these studies are not representative because they utilized a small number of subjects and did not have appropriate controls. Thus, evaluating whether prenatal zinc can reverse the impairments found in our rat model of autism would be interesting.

LPS and zinc can induce reproductive injuries, such as reduced litter size [8,19]. Thus, we evaluated the effects of LPS and zinc on female reproductive performance. Moreover, because impaired communication between children and their mothers is a typical symptom of autism [1], we evaluated ultrasonic vocalizations in isolated pups. We also evaluated the plasma levels of BDNF in rat offspring prenatally exposed to LPS and zinc because it is considered an important biomarker of autism [23]. Recently, Ricci et al. [2] showed that autistic patients presented elevated serum levels of BDNF, regardless of age, gender, and severity of the disorder, compared with controls. Other studies reported similar findings in plasma [24], serum [25], postmortem brain [26], and neonatal cord blood [27] samples from autistic patients.

2. Materials and methods

2.1. Ethics statement

This study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the School of Veterinary Medicine, University of São Paulo, Brazil (permit no. 2824/2012). All efforts were made to minimize suffering. The experiments were performed in accordance with good laboratory practice protocols and with quality assurance methods.

2.2. Animals

Fifteen pregnant Wistar rats between 12 and 13 weeks of age and weighing 226–266 g were used. Rat housing, the nutritional conditions, the determination of GD0, and the handling and care of dams were the same as those previously described by our group [9]. The dams were randomly divided into three groups ($n = 5$ per group). Two young male offspring (postnatal day [PND] 11) from each litter were used for the ultrasonic vocalization test ($n = 10$ per group). Two other adult male offspring (PND 68–72) from each litter were used for BDNF analysis ($n = 8$ per group, n randomly reduced according to methodological needs) We used two different offsprings for each experiment to minimize potential confounding factors associated with litter effects [28] and because maternal isolation required for ultrasonic vocalization test affects neurodevelopment and adult analyses [29]. All of the experiments were performed between 1:30 PM and 2:00 PM to minimize the effects of circadian rhythms.

2.3. Treatments

The LPS solution was described previously [9]. LPS was administered i.p. to pregnant dams at a dose of 100 μg/kg on GD9.5. One hour after LPS exposure, the dams also received sterile saline (SAL; 0.9% NaCl, 0.2 ml/100 g, subcutaneously [s.c.] in the nape of the neck) because saline was used as the vehicle for both LPS and zinc. This group was called LPS + SAL.

Another group received LPS (100 μg/kg on GD9.5, i.p.) and then zinc sulfate (zinc sulfate heptahydrate, $ZnSO_4$, Sigma, St. Louis, MO, USA, cat. no. Z0635; 2 mg/kg in 0.9% saline, s.c., in the nape of the neck) 1 h later, based on the findings of Coyle's group [30]. This group was called LPS + Zn. A s.c. zinc injection induces an immediate and consistently reproducible increase in plasma zinc that peaks at levels that are four- to five-fold higher than normal 2 h after injection and return to normal by 12 h [31]. No evidence has been reported that these plasma zinc levels have a detrimental effect on pregnancy outcome [20]. The recovery of normal zinc levels 12 h after s.c. zinc injection coincides with the period of increased levels of cytokines after LPS exposure [32,33]. The zinc solution was always prepared on the day of administration.

The control group consisted of pregnant rats that received only sterile saline on GD9.5 (0.2 ml/100 g, i.p.) and an additional saline injection after 1 h (0.2 ml/100 g, s.c.). This group was called SAL + SAL.

2.4. Reproductive performance

The dams were allowed to give birth and nurture their offspring normally. Gestation length from GD0 was evaluated. The day of birth was recorded as PND1. No handling was performed on PND1, because it is a known fact that PND1 is a critical period of stress for the dams. Handling on PND1 can lead to infanticide and cannibalism. On PND2, the number of males, females and total rat pups born per litter, and the total offspring weight were measured. Immediately after weighing, eight offspring (four males and four females) were randomly selected for the following studies. No cross-fostering procedure was used. Litters with fewer than eight pups were culled. Moreover, dams were weighed on GD9.5 and PND2 to estimate their weight gain during pregnancy. The pups remained with each dam until weaning (PND21). On PND21, littermates were separated and cohoused by sex under the same conditions as their parents.

2.5. Ultrasonic vocalization

Impaired communication of the pups with their mothers, a typical symptom of autism [1], was assessed by ultrasonic vocalization, validated for rat model of autism [9,34]. Rodent pups emit vocalizations (band frequency of 30–50 kHz) when isolated from their mothers, and these vocalizations are thought to solicit maternal interactions [35,36]. On PND 11, immediately after the pups were isolated from their nests and mothers, they were individually placed in a polypropylene cage ($30 \times 20 \times 12$ cm) and brought to a testing room at a controlled temperature of 22 °C ± 2 °C that was separate from the housing room. PND 11 was chosen because (1) ultrasonic vocalization is relatively temperature independent during the second week of life compared with the first week, (2) high within-litter variability in call emission is observed at this time point, and (3) substantial evidence indicates intraindividual stability in call emission at this time point [37,38]. Ultrasonic vocalizations were detected using Ultravox software (Noldus Information Technology, Leesburg, VA, USA) with a filter and ultrasonic microphone that was tuned to a range centered at 40 kHz and placed 8 cm away from the cage floor. The automatically recorded parameters during the 5-min session included the number of vocalizations, total time of vocalizations, mean vocalization duration, maximal vocalization duration, total silence duration, mean silence duration interval, and maximal silence duration interval. The durations were recorded in seconds.

2.6. BDNF analysis

Trunk blood was collected from adult offspring (PND68-72) after decapitation in conical tubes that contained ethylenediaminetetraacetic acid. The samples were centrifuged (10 min, 1000 g, 17 °C) and plasma was obtained. Plasma BDNF levels were determined in duplicate using the enzyme-linked immunosorbent assay according to the manufacturer's instructions (cat. no. G7610, Promega, Madison, WI, USA). We evaluated free mature BDNF (i.e., non-acidified samples) and total free BDNF (i.e., acid-treated and neutralized samples, which is the pro-form of BDNF, together with mature BDNF). Mature BDNF is the active form of this neurotrophin, which binds to the TrkB receptor [39].

Table 1
Reproductive performance. Effects of prenatal LPS (100 μg/kg) and zinc ($ZnSO_4$; 2 mg/kg) exposure on gestational day 9.5 on reproductive parameters of dams and their rat offspring. SAL + SAL, prenatal saline injection and another saline injection after 1 h; LPS + SAL, prenatal LPS injection and a saline injection after 1 h; LPS + Zn, prenatal LPS injection and zinc injection after 1 h ($n = 5$ dams/group; data are expressed as mean ± SEM).

Parameters	SAL + SAL group	LPS + SAL group	LPS + Zn group
Gestation length (days)	22.00 ± 0.00	22.00 ± 0.00	22.00 ± 0.00
Number of pups born	10.40 ± 0.68	7.00 ± 1.09*##	12.00 ± 0.55
Number of males born	6.20 ± 0.66	4.20 ± 0.73	5.20 ± 0.86
Number of females born	4.20 ± 1.07	2.80 ± 0.97#	6.80 ± 0.49
Dam weight (g) on GD9.5	241.6 ± 4.36	252.3 ± 5.49	246.9 ± 4.47
Dam weight gain (g, PND2-GD9.5)	4.84 ± 1.48	2.52 ± 5.74	3.40 ± 2.78

*$p < 0.05$, compared with SAL + SAL group; #$p < 0.05$ and ##$p < 0.01$, compared with LPS + Zn group (one-way ANOVA followed by Bonferroni test).

2.7. Statistical analysis

Homogeneity was verified using Bartlett's test. Normality was verified using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used to compare parametric data. The results are expressed as mean ± SEM. In all cases, the results were considered significant at $p < 0.05$.

3. Results

One-way ANOVA demonstrated that prenatal LPS and zinc influenced reproductive performance of dams (Table 1). There were differences for total number of pups born per litter ($F(2/27) = 9.98$, $p = 0.0028$). The multiple comparisons test revealed that prenatal LPS exposure (LPS + SAL) reduced offspring number compared to the control group (SAL + SAL; $p < 0.05$). Treatment with zinc prevented the offspring number reduction of dams exposed to LPS (LPS + Zn vs. LPS + SAL, $p < 0.01$) to the same levels as the control group. We also found differences in the number of females born ($F(2/27) = 5.33$, $p = 0.0221$). Interestingly, posttreatment with zinc (LPS + Zn) elevated the number of females born, compared with the LPS + SAL group ($p < 0.05$), without differences between SAL + SAL and LPS + SAL groups. Other reproductive parameters did not present statistically significant differences, i.e., no evidence was found that prenatal LPS and zinc exposure did not interfered with gestation length, number of male pups born, offspring weight, dam weight, and dam weight gain during pregnancy ($p > 0.05$ for all cases).

In the ultrasonic vocalization test, the one-way ANOVA revealed significant differences in the mean silence duration interval ($n = 10$ per group; $F(2/27) = 4.42$, $p = 0.0219$; Fig. 1). The multiple-comparison test revealed that prenatal LPS exposure (LPS + SAL group) increased the mean silence duration compared with the control group (SAL + SAL; $p < 0.05$). Posttreatment with zinc reduced the mean silence duration in rats prenatally exposed to LPS (LPS + Zn group vs. LPS + SAL, $p < 0.05$) to the same levels as the control group. The one-way ANOVA also revealed significant differences in the maximal silence duration interval ($n = 10$ per group; $F(2/27) = 3.88$, $p = 0.0330$). The multiple-comparison test revealed that prenatal LPS exposure (LPS + SAL group) increased the maximal silence duration compared with the control group (SAL + SAL; $p < 0.05$). Posttreatment with zinc reduced the maximal silence duration in rats prenatally exposed to LPS (LPS + Zn group vs. LPS + SAL, $p < 0.05$) to the same levels as the control group. However, no differences in the number of vocalizations ($n = 10$ per group; $F(2/27) = 0.06$, $p = 0.9365$) were found among the three groups. The total time of vocalization, mean vocalization duration, maximal vocalization duration, and total silence duration were also not different among the three groups ($n = 10$ per group; $p > 0.05$ in all cases, data not shown).

As shown in Fig. 2, the one-way ANOVA revealed significant differences in free mature BDNF ($n = 8$ per group; $F(2/21) = 5.29$, $p = 0.0137$). The multiple-comparison test revealed that plasma levels of free mature BDNF increased in the LPS + SAL group compared with the control group (SAL + SAL; $p < 0.05$). Posttreatment with zinc reduced the elevation of BDNF induced by LPS exposure (LPS + Zn group vs. LPS + SAL, $p < 0.05$) to the same levels as the control group. However, no differences in total free BDNF levels were found among the three groups ($n = 8$ per group; $F(2/21) = 2.48$, $p = 0.1080$).

4. Discussion

Prenatal infection/inflammation on GD 9.5 falls within a critical period for brain organogenesis. Infections associated with immunological events during the early/middle fetal stages (e.g., GD 8–10 in rats and mice) might have a stronger impact on neurodevelopment than infections that occur during late-stage pregnancy. Maternal immune activation during early/middle pregnancy may interfere with cell proliferation, differentiation, migration, target selection, and synapse maturation, eventually leading to multiple brain and behavioral abnormalities in adulthood [40–43]. Previous data from our group corroborated that GD 9.5 is a critical period. We showed that prenatal treatment with LPS on GD 9.5 in rats induced short- and long-term reproductive, behavioral, and neuroimmune impairments in the offspring [8–10,44–46].

Prenatal LPS exposure impaired the reproductive performance of rats. Prenatal LPS reduced the number of offspring born compared to the control group. Our previous studies have already shown a toxic effect of LPS in the reproductive performance, including injuries in the placental tissue and higher post-implantation loss [10]. Prenatal LPS can also induce spontaneous abortion, embryo resorption and intrauterine mortality [47,48].

Posttreatment with zinc prevented the reduction of the offspring number of dams exposed to LPS, restoring reproductive success similar to that found in controls. Accordingly, a study conducted with pregnant women in New Jersey have shown that pregnancy with low zinc diet

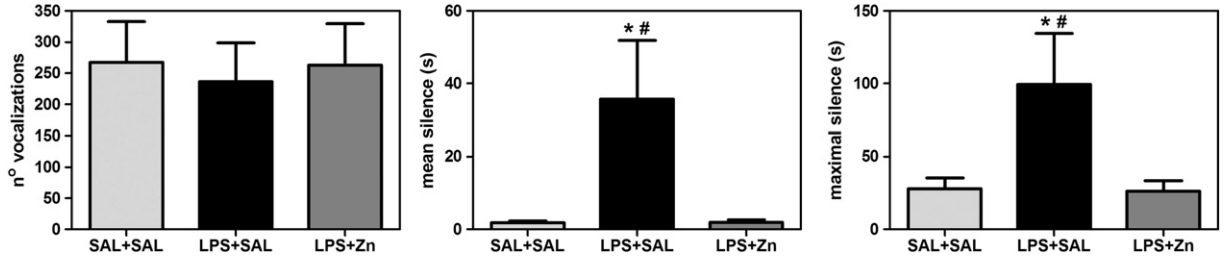

Fig. 1. Ultrasonic vocalizations of the offspring. Effects of prenatal LPS (100 μg/kg) and zinc ($ZnSO_4$; 2 mg/kg) exposure on gestational day 9.5 on ultrasonic vocalizations in infant male rat offspring. SAL + SAL, prenatal saline injection and another saline injection after 1 h; LPS + SAL, prenatal LPS injection and a saline injection after 1 h; LPS + Zn, prenatal LPS injection and zinc injection after 1 h ($n = 10$ rats/group). *$p < 0.05$, compared with SAL + SAL group; #$p < 0.05$, compared with LPS + Zn group (one-way ANOVA followed by Bonferroni test). The data are expressed as mean ± SEM.

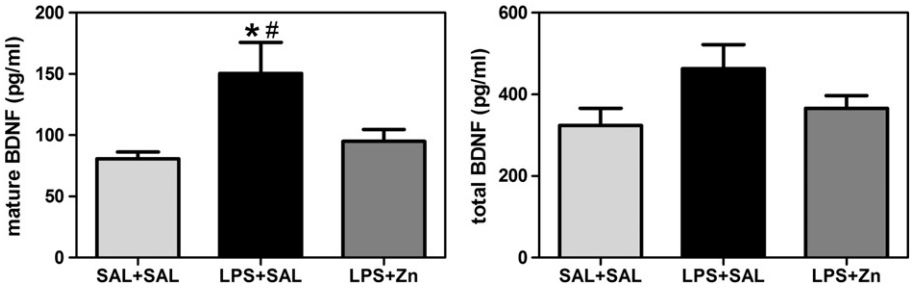

Fig. 2. BDNF levels of the offspring. Effects of prenatal LPS (100 μg/kg) and zinc ($ZnSO_4$; 2 mg/kg) exposure on gestational day 9.5 on plasma free mature and total free BDNF levels in adult male rat offspring. SAL + SAL, prenatal saline injection and another saline injection after 1 h; LPS + SAL, prenatal LPS injection and a saline injection after 1 h; LPS + Zn, prenatal LPS injection and zinc injection after 1 h ($n = 8$ rats/group. $^{*}p < 0.05$, compared with SAL + SAL group; $^{\#}p < 0.05$, compared with LPS + Zn group (one-way ANOVA followed by Bonferroni test). The data are expressed as mean ± SEM.

induced reproductive impairments, compared to women fed properly with zinc [49]. Proper transfer of zinc to the fetus during pregnancy is dependent on maintaining the normal concentration maternal zinc; in other words, there are no specific sites of zinc stock to compensate the momentary deficiency in maternal zinc [19,50]. Smoking, alcohol abuse and stressful responses to trauma and infections can reduce maternal concentration of zinc, being zinc supplementation prudent for women during pregnancy [50]. The oral administration of zinc to volunteers reduced reproductive impairments both in mother and fetus; zinc was well tolerated and accepted by patients, without inducing adverse effects [51].

In other mammals, the process seems to be similar. Coyle and colleagues demonstrated that LPS on GD8 induced a 40% decrease of plasma levels of zinc in mice. LPS induced teratogenesis, and zinc s.c. injection or dietary zinc supplementation of dams prevented these injuries [19,20,52].

According to Coyle et al. [19], control (saline) group that received zinc supplementation presented no reproductive performance impairments in maternal weight, number of litters, pup weight, and resorptions, although the litter size was slightly reduced compared with the control group. Thus, considering these reproductive results, we would not suggest prenatal zinc administration during gestation without infectious or inflammatory processes.

The fact that zinc treatment increased the number of females born compared to the data of the LPS group was extensively searched in the literature to verify whether this sexually dimorphic effect has been described, and if there is some explanation for this phenomenon. We did not find any similar report of sexually dimorphic effects induced by zinc. Thus, further studies are needed to verify the reproducibility of these data and elucidate the mechanisms involved in the dimorphism induced by prenatal zinc.

Pups that were prenatally exposed to LPS spent longer periods without emitting 40 kHz vocalizations to their mothers (i.e., prenatal LPS reduced vocal solicitations by infant offspring to their mothers). The 40 kHz ultrasonic vocalization frequency is used by pups when they are separated from their mothers to request the presence of the mother [53]. The present results showed that prenatal LPS exposure impaired communication in male pups. Impaired communication between children and their mothers is one of the main symptoms of autism [1]. The test of 40 kHz ultrasonic vocalization is validated for rat models of autism [9,34]. Thus, prenatal LPS exposure induced autistic-like behavior in rats.

Prenatal zinc administration prevented the communication impairment induced by LPS exposure to the same levels as controls. Therefore, prenatal zinc prevented autistic-like behavior in rats.

According to Caulfield et al. [54], prenatal zinc supplementation without inflammatory processes does not influence cognitive and social skills, as well as behavioral development. Thus, it seems that prenatal zinc induces behavioral changes only after an inflammatory process (e.g., induced by LPS).

BDNF is a small protein found throughout central nervous system, and peripheral blood. It is a member of a family of secreted proteins known as neurotrophins. It regulates neuronal survival, morphology, development, and function and plays a critical role in synaptogenesis and synaptic plasticity [55]. We also found that prenatal LPS exposure elevated BDNF levels in rats similarly to autistic patients [2,24,25]. To our knowledge, the present study that found that prenatal LPS exposure elevated BDNF levels is the first rodent model of autism to reveal this similarity to autistic patients. In fact, early BDNF hyperactivity may play an etiological role in autism early in life. This hypothesis is supported by previous studies that reported increased blood and brain tissue BDNF levels in autism compared with normal controls [56].

Together with our previous studies, our model induced impaired communication and socialization, induced repetitive/restricted behavior, increased serum IL-1β levels [8–10], and increased plasma BDNF levels in rats. Therefore, prenatal LPS exposure induced autism-like effects in offspring, revealing that it is a robust rat model of autism.

Tsai [56] hypothesized a correlation between autism and BDNF levels. Increased BDNF levels in autistic patients may reflect a regional compensatory mechanism or develop as an intrinsic component of the disease process. In fact, brain growth is abnormal in autism, indicated by early brain overgrowth. Because BDNF plays a key role in regulating neuronal survival [55], early BDNF hyperactivity could result in the overgrowth of brain tissue [56].

We cannot forget to mention that although the majority of studies involving autism and BDNF reveal high levels of BDNF in autistic patients, regardless of age, gender, severity of the disorder, and type of sample [2,24–27,56], the results are sometimes contradictory. For example, there are data showing that mean levels of BDNF are significantly lower in autistic children 0–9 years old, indicating a delayed BDNF increase with development and not an increase in BDNF levels [57].

Both human and rat studies have demonstrated that BDNF levels in the blood reflect BDNF levels in the brain [57,58]. Thus, our data suggest a disturbance of brain BDNF. Incidentally, in addition to autistic-like behavior, we previously found that our model of prenatal LPS also resulted in striatal dopaminergic impairments in adult offspring, including reduced levels of tyrosine hydroxylase, dopamine, and its metabolites [9,44]. BDNF is involved in the survival and differentiation of dopaminergic neurons in the developing brain [2]. For example, repeated administration of stimulant drugs can cause permanent changes in dopamine levels and both transient and permanent alterations in BDNF and tyrosine hydroxylase expression, inducing long-term neuroadaptations of both BDNF and dopamine [59,60]. BDNF is robustly expressed in the mesolimbic pathway. The mesolimbic pathway is the anatomical substrate for intricate interactions between dopamine and BDNF [61]. We believe that the striatal dopaminergic and BDNF disturbances found after prenatal LPS exposure may be correlated, but this possibility requires further study.

Interestingly, we found a disturbance in free mature BDNF levels and not total free BDNF levels, which includes the BDNF precursor pro-

BDNF. However, both pro-BDNF and mature BDNF have been found to be abnormal in patients with autism [26,62]. Specifically, mature BDNF promotes spine formation, neuronal survival, and long-term potentiation [63,64].

In addition to demonstrating that the plasma levels of free mature BDNF were increased after prenatal LPS exposure, we also found that posttreatment with zinc reduced the elevation induced by LPS exposure to the same levels as the control group. Zinc is one of the most important trace elements in mammals, and it is required for many physiological processes, such as cell proliferation and differentiation, growth and development, and the regulation of enzymatic activity [65]. Some studies in the literature show that zinc administration may induce BDNF expression [66]. Yang and colleagues [67] showed that zinc supplementation reduced BDNF levels in the hippocampus by reducing BDNF-TrkB neurotrophic signaling. In fact, zinc was previously proposed to be a potential agent for the treatment of Rett syndrome [68], which is included in autism spectrum disorder [1]. However, we emphasize that the present study is likely the first to investigate zinc for the prevention/treatment of autism with regard to the BDNF pathway.

Yu et al. [69] demonstrated that pups of pregnant rats that were orally supplemented with zinc presented a decrease in hippocampal BDNF levels compared with control animals. Thus, considering the BDNF impairments induced by zinc, we would not suggest prenatal zinc administration during gestation without infectious or inflammatory processes.

The present findings revealed a potential beneficial effect of prenatal zinc administration for the prevention of autism. Importantly, epidemiological studies have correlated infections during pregnancy with a higher incidence of children diagnosed with autism [70]. Thus, by extrapolating to humans, we suggest that when the first signs of sickness behavior associated with an infection in pregnant women are perceived, such as those induced by LPS, zinc could be administered to prevent the development of autism in newborns.

5. Conclusions

In conclusion, prenatal LPS exposure on GD 9.5 impaired communication and increased plasma BDNF levels in rat offspring. This communication impairment and BDNF hyperactivity was similar to many studies of autistic patients [1,2,24,25,27,71]. Together with our previous studies [8,9] our model induced autistic-like behavioral, brain, and immune disturbances, demonstrating that it is a robust rat model of autism. We also found that posttreatment with zinc prevented the communication impairment and BDNF hyperactivity induced by LPS exposure. Thus, to our knowledge, this is the first study to reveal a potential beneficial effect of prenatal zinc administration for the prevention/treatment of autism with regard to the BDNF pathway. The reproductive parameters study corroborates the toxic effects of LPS during gestation, and the beneficial preventive effect of zinc in the reproduction. The present findings may contribute to a better understanding and prevention/treatment of autism and associated diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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