

ORIGINAL ARTICLE

Increased neuronal seizure activity correlates with excessive systemic inflammation in a rat model of severe preeclampsia

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Preeclampsia (PE), one of the most common disorders of pregnancy, is characterized by hypertension and albuminuria. In severe cases, PE results in eclampsia-like seizures. Studies have suggested that severe PE is related to an exaggerated systemic inflammatory response, which may increase sensitivity to seizures. In the current study, we investigated whether the seizure activity of neurons was enhanced under excessive systemic inflammation. We also sought to determine whether MgSO₄ could reduce the effects of systemic inflammation on seizure activity after electrical stimulation in a lipopolysaccharide (LPS)-induced model of PE. In addition to pregnancy outcomes, we analyzed biochemical parameters to ascertain whether our PE model was successful. Enzyme-linked immunosorbent assay analysis revealed that the levels of inflammatory cytokines (tumor necrosis factor (TNF)- α and interleukin (IL)-1 β) were significantly higher in the LPS-treated rats than in the untreated rats. After electrical stimulation, behavioral assessments showed that the LPS-treated rats that were not treated with MgSO₄ had the shortest latency period to develop a seizure and the longest seizure duration. The electroencephalographic (EEG) recordings in the hippocampus demonstrated that this group also had the highest EEG amplitude. MgSO₄ treatment significantly decreased both TNF- α and IL-1 β concentrations, increased the latency to develop a seizure, decreased the seizure duration and shortened the EEG amplitude. These results suggest that neuronal seizure activity and systemic inflammation are increased in severe PE. In addition, MgSO₄ treatment reduced systemic inflammation and seizure severity. We conclude that excessive systemic inflammation in PE promotes eclampsia seizures, which can be attenuated by MgSO₄ treatment.

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INTRODUCTION

Preeclampsia (PE) is a serious complication of pregnancy. Its clinical manifestations are maternal hypertension, albuminuria, liver and kidney dysfunction, fetal growth restriction and maternal and infant death in late pregnancy.¹ According to the World Health Organization's estimates of global incidence, >4 million live births are affected by PE each year, resulting in maternal deaths between 40 000 and 72 000.² Eclampsia is defined as the development of grand mal seizures in patients with gestational hypertension or PE.³ The risk of maternal death caused by eclampsia is 0–1.8% in developed countries and as high as 15% in the developing countries.^{4–7} PE and eclampsia continue to be a major cause of maternal and fetal morbidity and mortality, but their pathogenesis remains unclear.

Clinical data suggest that excessive systemic inflammatory is associated with PE.^{1,8} PE has been found to involve the activation of a large number of white blood cells,⁹ and inflammatory markers, such

as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and C-reactive protein, were increased at least twofold in PE patients compared with non-pregnant patients.^{8–12} (TNF- α infusion and low-dose lipopolysaccharide (LPS) infusion have been used to establish animal models of PE by inducing systemic inflammation in pregnant rats.¹⁰ Eclampsia is associated with the development of tonic-clonic seizures in PE patients.¹¹ Seizures are caused by synchronized aberrant electrical activity of neuronal populations owing to underlying hyperexcitability.^{12,13} Vezzani *et al.*¹⁴ showed that proinflammatory cytokines such as IL-1 β and TNF- α can enhance neuronal excitability and initiate spontaneous seizures. Key inflammatory mediators are also correlated with seizure frequency¹⁵; therefore, it is possible that a systemic inflammatory response is involved in the pathogenesis of PE and eclampsia.

Magnesium sulfate (MgSO₄) has been shown to have therapeutic effects in PE and reduces the release of acetylcholine in the

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neuromuscular junction, which causes the muscles to relax,¹⁶ increases the synthesis of prostacyclins, increases vasodilation by inhibiting angiotensin-converting enzyme¹⁷ and improves sedation of the nervous system.¹⁸ In addition, Dowling *et al.*¹⁹ found that administration of MgSO₄ inhibited LPS-induced excessive inflammation at the maternal–fetal interface. Other studies have also highlighted the anti-inflammatory effects of MgSO₄. Nuan-Yen's study²⁰ suggested that MgSO₄ could prevent the endotoxin-induced upregulation of inflammatory mediators *in vitro*²¹ and mitigate the inflammatory response in rats with endotoxemia.²² Because PE and eclampsia are often associated with abnormal maternal inflammation, parenteral MgSO₄ has been an important drug for treating severe PE.²³

In the present study, to investigate whether systemic inflammation correlates with seizure activity, we established a new eclampsia-like model using electrical stimulation. We measured systolic blood pressure (SBP), proteinuria, creatinine (Cr), blood urea nitrogen (BUN), alanine transaminase (ALT) and aspartate transaminase (AST) to determine whether the model was successful. The seizure activity of neurons in the eclampsia-like model was recorded and analyzed using behavioral methods and electroencephalography (EEG). Furthermore, we detected inflammatory markers to investigate the relationship of systemic inflammation with seizure activity. In addition, we explored the effects of MgSO₄ treatment on seizure activity and inflammatory responses.

MATERIALS AND METHODS

Animals

Experimental protocols were approved by the Committee on the Ethics of Animal Experiments of Guangzhou Medical University (Permit Number: 2012–50) and were in strict accordance with the recommendations of the NIH Guidelines (NIH Publications No. 8023, revised 1978) for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. A total of 45 female Sprague Dawley rats (weight, 210–250 g; 10–12 weeks old) were obtained from the Medical Experimental Animal Center of Guangdong, China. The rats were acclimatized to the laboratory conditions for 1 week prior to the start of the experiments. Each rat used in all the pregnancy groups was separately mated overnight. Day 0 of pregnancy was defined as the day when spermatozoa were found in a vaginal smear.²⁴ There were five experimental groups: the non-pregnant group (NP group, $n=12$), the non-pregnant group treated with LPS (NP+LPS group, $n=12$), the pregnant group (P group, $n=6$), the pregnant group treated with LPS (P+LPS group, $n=7$), and the pregnant group treated with LPS and MgSO₄ (P+LPS+Mg group, $n=8$).

Implantation of electrodes for electrical stimulation and EEG recording

For all surgeries, the rats were anesthetized with 10% chloral hydrate (3.5 ml kg⁻¹, i.p.). EEG electrodes were made using a section of insulated Nichrome wire (0.2 mm in diameter with uninsulated tips) that was soldered to a 080-1/8 inch stainless steel screw (RWD Life Science, Shenzhen, China), which was implanted and fixed to the skull using dental acrylic.²⁵ The free end of the wire was soldered to a SM-3P wiring terminal coupled to a stimulating electrode and the EEG recording equipment. A stereotaxic frame (RWD Life Science) was used to guide the electrodes to the correct location in the hippocampus. The electrode was implanted in the right dorsal hippocampus (3.8 mm behind the bregma, 3.9 mm right of the midline and 3.7 mm below the skull bone). After the scalps were sutured, the rats were individually housed in cages and allowed to recover for 7–10 days.

Rat model of PE

On gestational day (GD) 14, the rats in the P+LPS and NP+LPS groups were anesthetized and received 2 ml of LPS (1.0 µg kg⁻¹) infused through the tail vein with a pump at a rate of 2 ml h⁻¹ according to method described by

Fass *et al.*²⁶ On the same day, the rats in the NP and P groups were infused with 0.9% saline using a similar protocol.

Electrical stimulation and EEG recordings in the PE model

On GD 18, all rats received electrical stimulation. The rats were individually placed in a 20 × 35 × 25 cm³ box and allowed to freely explore for at least 10 min before the start of electrical stimulation. After 10 min, the rats received the electrical stimulations (0.2 mA, 20 Hz, 100 ms intervals, rectangular pulses). The behavioral characteristics of the seizures were classified and recorded according to Racine's staging: stage 1, mouth and facial movements; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing; and stage 5, rearing and falling. A grand mal seizure was referred to as a stage 5 motor seizure.²⁷ The latency to develop a stage 5 seizure was defined as the time from the start of the electrical stimulation to the occurrence of an evoked stage 5 seizure. The stimulation duration was defined as the time to develop a tonic–clonic seizure (to a maximum of 30 min). EEG data were continuously recorded during the procedure using a BL-420S Biological Functional System (Chengdu Taimeng Technologies, Chengdu, China).

MgSO₄ treatment

On GD 14, after LPS infusion, osmotic mini-pumps (Model 2ML1; Alzet Corporation, Palo Alto, CA, USA), which were loaded to continuously deliver MgSO₄ (60 mg kg⁻¹ day⁻¹),²⁸ were s.c. inserted into the mid-scapular region of each rat in the P+LPS+Mg group.

Measurement of biochemical parameters and cytokine analysis

On GDs 7, 13, 15, 17 and 19, SBP was measured between 0900 and 1200 hours using a non-invasive blood pressure system (Chengdu Taimeng Technologies). SBP measurements were performed three times in 1 day for each rat, and the mean values were recorded. On GD 16, the rats were placed into metabolic cages and urine samples were collected. Twenty-four-hour urine protein excretion was measured using an auto-analyzer (HITACHI automatic analyzer, 7600-020, HITACHI, Tokyo, Japan). On GD 18, prior to electrical stimulation, 2 ml of blood was collected from the intraorbital sinus of each rat, and the serum was separated for biochemical parameters and cytokine analysis. On GD 20, we repeated the cytokine analysis. On GD 21, the rats were anesthetized with a non-lethal dose of chloral hydrate and blood samples were collected. After the blood collection, the rats were killed. The pups and the placentas were removed through cesarean section and were counted, weighed and examined for resorption and malformation. Measurements of liver enzymes (ALT, AST), BUN and Cr were performed using an automatic analyzer (HITACHI 7600-020). The serum levels of cytokines (TNF-α and IL-1β) were determined using a highly sensitive enzyme-linked immunosorbent assay (eBioscience, San Diego, CA, USA). For each assay, the standards and samples were tested in duplicate.

Drugs and solutions

LPS (*E. coli*, 0.55:B5; Sigma Aldrich, St Louis, MO, USA) was dissolved in 0.9% saline, and a stock solution of 1 mg ml⁻¹ of LPS was prepared and stored at –20 °C. A 10% MgSO₄ solution was prepared and stored at 4 °C.

Statistical analysis

The normally distributed continuous data are expressed as the mean ± s.e.m. A one-way analysis of variance was used to compare the average values between the different groups, which was followed by the least significant difference *post-hoc* test. The rats were compared using chi-squared test or Fisher's exact test. All the statistical analyses were performed using the SPSS 13.0 software package (SPSS, Chicago, IL, USA). A P -value < 0.05 was considered statistically significant.

RESULTS

SBP was increased in the P+LPS group

To confirm that the rats in the P+LPS group developed PE-like symptoms, we analyzed the SBP of each rat. Before LPS administration, quantitative analysis (Figure 1) revealed that there were no significant differences between the groups on GDs 7 and 13 ($P > 0.05$).

After LPS administration, SBP significantly increased in the P+LPS group at GDs 17 (129 ± 3 mm Hg) and 19 (135 ± 4 mm Hg), compared with GDs 7 (112 ± 3 mm Hg), 13 (113 ± 4 mm Hg) and 15 (119 ± 2 mm Hg) ($P < 0.05$). In addition, SBP at GD 17 was significantly higher in the P+LPS group than in the NP (111 ± 2 mm Hg), NP+LPS (110 ± 3 mm Hg) and P (113 ± 2 mm Hg) groups ($P < 0.05$). SBP at GD 19 was also higher in the P+LPS group than in the NP (111 ± 2 mm Hg), NP+LPS (110 ± 3 mm Hg) and P (113 ± 2 mm Hg) groups ($P < 0.05$).

Changes in biochemical outcomes in groups without magnesium (Mg) treatment

To further ascertain whether the PE model was successful, we measured the levels of proteinuria, Cr, BUN, ALT and AST using an automatic analyzer (Table 1). The results showed that proteinuria was significantly higher in the P+LPS group (3.8 ± 0.5 mg per 24 h) than in the NP (2.3 ± 0.4 mg per 24 h), NP+LPS (2.4 ± 0.4 mg per 24 h) and P (2.6 ± 0.6 mg per 24 h) groups ($P < 0.05$). Furthermore, ALT levels were significantly higher in the P+LPS group (91 ± 11.3 U l⁻¹) than in the NP (64 ± 3.5 U l⁻¹) and NP+LPS (78 ± 6.7 U l⁻¹) groups ($P < 0.05$). Moreover, the AST level was higher in the P+LPS group (191 ± 14.3 U l⁻¹) than in the NP (117 ± 6.9 U l⁻¹), NP+LPS (121 ± 8.7 U l⁻¹) and P (131 ± 11.2 U l⁻¹) groups ($P < 0.05$). However, BUN and Cr levels were not significantly different between the P+LPS, NP, NP+LPS and P groups.

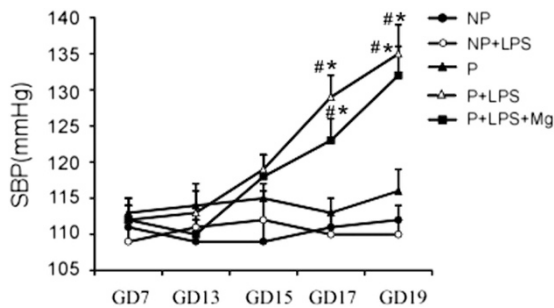


Figure 1 Changes in the mean systolic blood pressure (SBP) in all the five groups at 7, 13, 15 and 19 days after gestation. The values are presented as the mean \pm s.e.m. SBP was significantly increased in the P+LPS group compared with the NP, NP+LPS and P groups on gestation days (GDs) 17 and 19 ($*P < 0.05$). Following LPS administration, SBP was also increased in the P+LPS group on GDs 17 and 19 compared with GDs 7 and 13. SBP in the P+LPS+Mg group exhibited a similar pattern as the P+LPS group ($\#$, $*P < 0.05$). NP = non-pregnant, NP+LPS = non-pregnant+LPS, P = pregnant, P+LPS = pregnant+LPS, P+LPS+Mg = pregnant+LPS +MgSO₄.

Table 1 Biochemical parameters in all the experimental groups on day 18 after gestation

Group (n)	Proteinuria (mg per 24 h)	Cr (μ mol l ⁻¹)	BUN (mmol l ⁻¹)	ALT (U l ⁻¹)	AST (U l ⁻¹)
NP (12)	2.3 \pm 0.4	35.7 \pm 2.1	6.1 \pm 0.9	64 \pm 3.5	117 \pm 6.9
NP+LPS (12)	2.4 \pm 0.4	34.1 \pm 2.3	6.2 \pm 0.9	78 \pm 6.7	121 \pm 8.7
P (6)	2.6 \pm 0.6	36.5 \pm 2.6	6.5 \pm 0.8	82 \pm 8.4	131 \pm 11.2
P+LPS (7)	3.8 \pm 0.5 ^a	32.9 \pm 2.7	7.5 \pm 1.0	91 \pm 11.3 ^b	191 \pm 14.3 ^a
P+LPS+Mg (8)	3.5 \pm 0.5	31.4 \pm 1.8	7.1 \pm 0.7	89 \pm 7.6	176 \pm 12.9

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cr, creatinine; NP, non-pregnant; NP+LPS, non-pregnant+lipo polysaccharide; P, pregnant; P+LPS, pregnant+lipo polysaccharide; P+LPS+Mg, pregnant+lipo polysaccharide+magnesium sulfate.

^a $P < 0.05$, P+LPS vs. NP, NP+LPS and P.

^b $P < 0.05$, P+LPS vs. NP and NP+LPS.

Poor pregnancy outcomes in the P+LPS group

PE is also characterized by poor pregnancy outcomes. Therefore, we measured the number of live fetuses, the percentage of resorbed fetuses, the fetal weight and the placental weight (Table 2). The results showed that the percentage of fetal resorption was increased by 79.2% in the P+LPS group (24%) compared with the P group (5%). Furthermore, the fetal weight was lower in the P+LPS group (3.97 ± 0.04 g) than in the P group (4.31 ± 0.12 g) ($P < 0.05$). There were no significant differences in the number of live fetuses and the placental weight between the P+LPS and P groups.

Increased seizure activity in the P+LPS group after electrical stimulation as detected using behavioral methods

The electrical stimulation-induced seizure behavior was assessed according to Racine's scale.^{27,29} After electrical stimulation, all the pregnant rats that were infused with either 0.9% saline or LPS developed stage 5 seizures, whereas only 58.3% of non-pregnant rats developed a full motor seizure. The latency to develop tonic-clonic seizures was significantly shorter in the P+LPS group (3.3 ± 0.7 min) than in the NP (10.6 ± 2.1 min) and NP+LPS (11.3 ± 1.8 min) groups ($P < 0.05$, Figure 2a). Furthermore, the seizure duration was significantly longer in the P+LPS rats (21.5 ± 2.1 s) than in the NP (13.4 ± 1.2 s), NP+LPS (14.1 ± 1.4 s) and P (16.7 ± 1.7 s) groups ($P < 0.05$, Figure 2b).

EEG neuronal seizure activity increased in the P+LPS group after electrical stimulation

Because an abnormal EEG can help to predict the risk of seizure recurrence, EEG has been proposed as part of the initial neurological diagnostic evaluation in pregnant patients.³⁰ EEG recordings were used to show the seizure activity when the rats developed clinical tonic-clonic convulsions and revealed changes in seizure activity before and after electrical stimulation in the different groups. No trace of evoked seizures was found in the five groups before electrical stimulation (Figures 3a–e). After stimulation, dramatic seizures were observed in the EEG traces in the NP (Figure 3a-1), NP+LPS (Figure 3b-1), P (Figure 3c-1) and P+LPS groups (Figure 3d-1); all of the seizures lasted for 4–5 s. Furthermore, the amplitude of the seizure traces was twice as high as the traces before electrical stimulation.

Statistical analysis revealed that EEG amplitude increased significantly after electrical stimulation in each group compared with the prestimulation recordings (NP: 90.54 ± 12.5 vs. 23.45 ± 2.56 μ V; NP+LPS: 89.68 ± 13.38 vs. 25.17 ± 2.12 μ V; P: 92.27 ± 11.17 vs. 27.93 ± 2.78 μ V; P+LPS: 107.74 ± 14.29 vs. 26.07 ± 2.89 μ V; $P < 0.01$). There were no significant differences in the EEG amplitude between the P+LPS group and the NP, NP+LPS and P groups after electrical stimulation (Figure 3f).

Table 2 Pregnancy outcomes in different groups on gestation day 21

Group (n)	Number of live fetuses	Percentage of resorbed fetuses	Fetal weight (g)	Placental weight (g)
P (6)	9.5 ± 1.1 (57)	5.0% (3/60)	4.31 ± 0.12	0.56 ± 0.03
P+LPS (7)	8.2 ± 0.5 (57)	24.0% (18/75)	3.97 ± 0.04*	0.53 ± 0.03
P+LPS+Mg (8)	9.3 ± 0.6 (74)	11.9% (10/84)	4.12 ± 0.06	0.52 ± 0.02

Abbreviations: P, pregnant; P+LPS, pregnant+lipopolysaccharide; P+LPS+Mg, pregnant+lipopolysaccharide+magnesium sulfate. * $P < 0.05$, P+LPS vs. p.

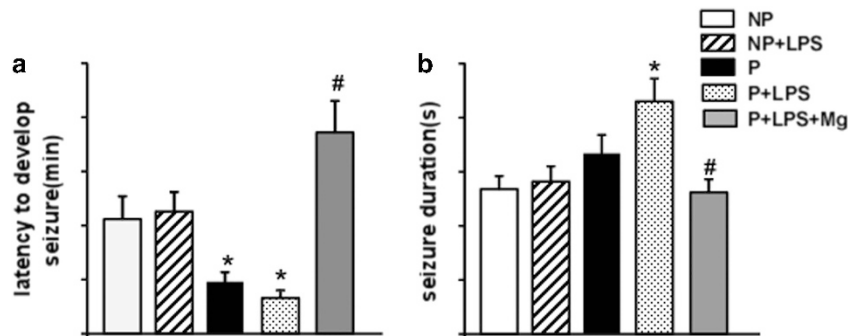


Figure 2 Changes in the latency to develop grade 5 seizures and the seizure duration in all the experimental groups. (a) Compared with the NP and NP+LPS groups, the latency to develop grade 5 seizures was significantly decreased in the P and P+LPS groups (* $P < 0.05$). However, MgSO₄ treatment increased the latency in the P+LPS+Mg group compared with the other four groups (# $P < 0.05$). (b) Compared with NP and the NP+LPS groups, the seizure duration was significantly increased in the P and P+LPS groups (* $P < 0.05$). In addition, the seizure duration was longer in the P+LPS group than in the P group. MgSO₄ treatment significantly decreased the seizure duration in the P+LPS+Mg group compared with the P and the P+LPS groups (# $P < 0.05$).

Increased inflammatory cytokines in the PE model

The concentrations of TNF- α and IL-1 β were measured before and after electrical stimulation (Table 3). TNF- α was not detected in the NP and the NP+LPS groups before and after stimulation. However, the level of TNF- α was approximately 30% higher in the P+LPS group (23.6 ± 4.01 pg ml⁻¹) than in the P group (16.99 ± 2.81 pg ml⁻¹). After stimulation, the TNF- α levels were 25% higher in the P+LPS group (24.02 ± 4.72 pg ml⁻¹) than in the P group (18.42 ± 2.45 pg ml⁻¹); however, these differences were not statistically significant. The IL-1 β levels were significantly higher in the P+LPS group (82.56 ± 8.19 pg ml⁻¹) before stimulation than in the NP (40.92 ± 4.64 pg ml⁻¹), NP+LPS (43.35 ± 5.49 pg ml⁻¹) and P groups (57.91 ± 6.21 pg ml⁻¹) ($P < 0.05$). After stimulation, the IL-1 β levels were also higher in the P+LPS group (88.94 ± 7.91 pg ml⁻¹) than in the NP (44.84 ± 5.49 pg ml⁻¹), NP+LPS (41.18 ± 4.35 pg ml⁻¹) and P (60.08 ± 7.16 pg ml⁻¹) groups ($P < 0.05$). However, there were no differences in TNF- α and IL-1 β levels within each group after electrical stimulation.

The effect of MgSO₄ on the PE and seizure models

The serum magnesium concentration was significantly higher in the P+LPS+Mg group (0.86 ± 0.24 mmol l⁻¹) than in the groups that did not receive MgSO₄ treatment (the P group, 0.63 ± 0.09 mmol l⁻¹; and the P+LPS group, 0.61 ± 0.12 mmol l⁻¹; $P < 0.05$). There was no significant difference in SBP between the P+LPS+Mg and P+LPS groups (Figure 1). Furthermore, there were no differences in the levels of proteinuria, Cr, BUN, ALT and AST in the P+LPS+Mg group compared with the PE group (proteinuria: 3.5 ± 0.5 vs. 3.8 ± 0.5 mg per 24 h; Cr: 31.4 ± 1.8 vs. 32.9 ± 2.7 μ mol l⁻¹; BUN: 7.1 ± 0.7 vs. 7.5 ± 1.0 mmol l⁻¹; ALT: 89 ± 7.6 vs. 91 ± 11.3 U l⁻¹; and AST: 176 ± 12.9 vs. 191 ± 143 U l⁻¹; Table 1). In addition, the number of live fetuses (9.3 ± 0.6), fetal weight (4.12 ± 0.06 g) and placental weight (0.52 ± 0.02 g) were not significantly different after MgSO₄ treatment

compared with the values in the P+LPS group. However, the percentage of resorbed fetuses decreased by approximately 50% in the P+LPS+Mg group (11.9%) compared with the PE group (24%) (Table 2).

After stimulation, the rats in the P+LPS group developed seizures. MgSO₄ administration decreased the degree of the seizures. The latency to develop stage 5 seizures was significantly longer in the P+LPS+Mg group (18.6 ± 2.9 min) than in the P+LPS group (3.3 ± 0.7 min) ($P < 0.01$, Figure 2a). In addition, seizure duration was significantly shorter in the P+LPS+Mg group (13.1 ± 1.2 s) than in the P+LPS group (21.5 ± 2.1 s) ($P < 0.01$, Figure 2b). Furthermore, EEG was used to record the neuronal seizure activity. After stimulation, the spikes in the trace, which indicates a lower EEG amplitude, was shorter in the P+LPS+Mg group (78.25 ± 11.57 μ V) than in the P+LPS group (107.74 ± 14.29 μ V, $P < 0.05$). The total seizure duration was slightly shorter after MgSO₄ treatment (Figure 3e-1). However, there were no significant differences between the two groups before the electrical stimulation. In the P+LPS+Mg group, EEG amplitude was also significantly increased after stimulation (78.25 ± 11.57 μ V) compared with the unstimulated conditions (22.48 ± 2.91 μ V, $P < 0.01$, Figure 3f).

Inflammatory cytokines were also reduced by MgSO₄ treatment. Before stimulation, the TNF- α levels were lower in the P+LPS group (23.36 ± 4.01 pg ml⁻¹) than in the P+LPS+Mg group (17.25 ± 3.84 pg ml⁻¹). After stimulation, the TNF- α levels were decreased in the P+LPS group (24.02 ± 4.72 pg ml⁻¹) compared with the P+LPS+Mg group (18.96 ± 3.15 pg ml⁻¹). However, these changes were not statistically significant. Before stimulation, the IL-1 β levels were lower in the P+LPS+Mg group (63.19 ± 7.24 pg ml⁻¹) than in the PE group (82.56 ± 8.19 pg ml⁻¹, $P < 0.05$). After stimulation, the IL-1 β level was also significantly lower in the P+LPS+Mg group (72.24 ± 6.25 pg ml⁻¹) than in the PE group (88.94 ± 7.91 pg ml⁻¹, $P < 0.05$, Table 3).

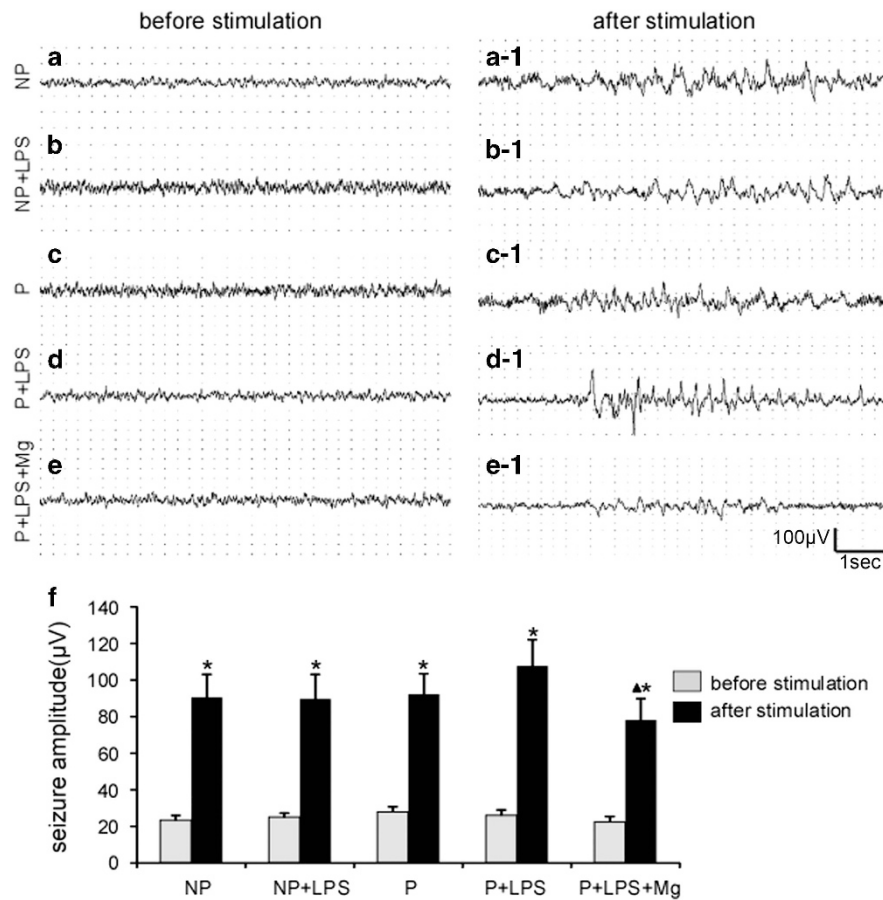


Figure 3 EEG recordings before and after electrical stimulation in the different groups. Before electrical stimulation, EEG traces showed that there were no convulsive seizures in all the five groups (a–e). Electrical stimulation induced seizures in the NP, NP+LPS, P and P+LPS groups, and the amplitudes of the EEG traces were two to three times higher compared with the EEG traces before stimulation (a-1, b-1, c-1 and d-1). MgSO₄ treatment significantly decreased the seizure amplitude (e-1). (f) Histogram of the EEG amplitude. The EEG amplitudes were significantly increased after electrical stimulation in the NP, NP+LPS, P and P+LPS groups (**P*<0.01). A comparison of the EEG amplitudes between the groups after electrical stimulation showed that the amplitude was significantly decreased in the P+LPS+Mg group compared with the P+LPS group (▲**P*<0.05).

Table 3 TNF-α and IL-1β concentrations in all the experimental groups

Group (n)	Serum level of TNF-α (pg ml ⁻¹)		Serum level of IL-1β (pg ml ⁻¹)	
	Before stimulation	After stimulation	Before stimulation	After stimulation
NP (12)	Non-detectable	Non-detectable	40.92 ± 4.64	44.84 ± 5.49
NP+LPS (12)	Non-detectable	Non-detectable	43.35 ± 5.49	41.18 ± 4.35
P (6)	16.99 ± 2.81	18.42 ± 2.45	57.91 ± 6.213 ^a	60.08 ± 7.16 ^a
P+LPS (7)	23.36 ± 4.01	24.02 ± 4.72	82.56 ± 8.19 ^b	88.94 ± 7.91 ^b
P+LPS+Mg (8)	17.25 ± 3.84	18.96 ± 3.15	63.19 ± 7.24	72.24 ± 6.25

Abbreviations: IL, interleukin; NP, non-pregnant; NP+LPS, non-pregnant+lipopolysaccharide; P, pregnant; P+LPS, pregnant+lipopolysaccharide; P+LPS+Mg, pregnant+lipopolysaccharide+magnesium sulfate; TNF, tumor necrosis factor.
^a*P*<0.05, P vs. NP and NP+LPS.
^b*P*<0.05, P+LPS vs. NP, NP+LPS, P and P+LPS+Mg.

DISCUSSION

PE and eclampsia models

PE is a common disease during pregnancy; however, the mechanisms underlying its pathogenesis remain unclear. Therefore, animal models of PE are necessary to study the etiology of PE and explore therapeutic treatment interventions. Several key events have been shown to be involved in the early development of PE, including insufficient spiral artery remodeling and higher plasma levels of soluble fms-like tyrosine kinase-1. Higher plasma levels of soluble fms-like tyrosine kinase-1 has

been shown to antagonize vascular endothelial growth factor, which causes abnormal spiral artery remodeling^{31–33}; therefore, vascular endothelial growth factor inhibitors have been used as a model of PE.^{34,35} In addition, based on two predominant theories of PE (the locally chronic uterine placenta ischemia and hypoxia theory and the excessive activation of the maternal immune system theory), additional animal models of PE have been developed. These include uteroplacental ischemia models,³⁶ nitric oxide synthase inhibition models,^{37,38} inflammation-related models (such as LPS infusion)³⁹

and TNF- α infusion models.⁴⁰ Unfortunately, only a few of the PE models lead to the development of eclampsia-like seizures. We have previously used pentylenetetrazol treatment to induce eclampsia-like seizures in pregnant rats treated with low-dose LPS.⁴¹ Johnson *et al.*⁴² combined reduced uteroplacental perfusion pressure with a high-cholesterol diet to develop an eclampsia model. In the present study, we successfully induced eclampsia-like seizures using electrical stimulation in the hippocampus of PE rats. This model excludes the effects of external chemical agents and directly reflects the neuronal firing activity observed in PE.

Inflammatory mechanisms might participate in seizure occurrence in PE after electrical stimulation

The outcomes of PE have been found to be associated with systemic inflammation,^{43–46} which includes increases in serum levels of circulating proinflammatory cytokines, such as TNF- α , IL-1 and IL-6.^{47,48} Systemic inflammation can be rapidly activated by LPS administration in experimental models, which subsequently increases the levels of inflammatory cytokines (TNF- α or IL-1 β) and oxygen-free radicals.^{8,49} In the present study, we administered LPS to pregnant rats to promote the development of PE and found that the serum levels of IL-1 β significantly increased in the PE group compared with the NP, NP+LPS and P groups. Furthermore, TNF- α levels also increased by 30% compared with the P group. These results are consistent with previous findings on cytokines in PE^{41,50–52} which have shown that direct or indirect endothelial dysfunction may result in the excessive production of cytokines, reduction in angiogenic processes and renal dysfunction.^{48,53} More importantly, we investigated whether seizure susceptibility increased significantly in the PE group, corresponding to the increased inflammation after electrical stimulation. First, behavioral assessments revealed that seizure activity was the strongest in the PE group after electrical stimulation. Second, EEG recordings demonstrated that the EEG amplitude was the longest in the PE group after electrical stimulation; however, there was no significant difference between the PE group and the NP, NP+LPS and P groups. These results indicated that the PE group was more inclined to develop seizures. In addition, we observed increased signs of inflammation in the PE group, which suggests that excessive inflammation decreased seizure sensitivity. It is worth noting that the serum levels of IL-1 β and TNF- α were significantly higher in the pregnancy groups than in the NP and NP+LPS groups. The latency to develop stage 5 seizures was also decreased in the pregnancy groups compared with the NP and NP+LPS groups. Seizure duration and EEG amplitudes were also increased in the P group, although there were no significant differences between the P group and the NP and NP+LPS groups. These results suggest that during normal pregnancy, cytokines are also elevated and promote seizure activity. The findings are consistent with the findings by Cipolla *et al.*,⁵⁴ who found that increased serum levels of cytokines during pregnancy could induce neuroinflammation and seizure activity. In fact, during normal pregnancy, the immune system changes to meet the demands of the growing fetus. As a result, high levels of cytokines are released into the circulation and may have an important role in the development of seizures later in gestation.^{55,56} These studies further suggest that increased systemic inflammation is related to seizure sensitivity.^{57,58} Systemic inflammation has been suggested to have transient or long-term effects on seizure susceptibility by inducing a mirror inflammatory response in the brain through microglial activation.^{59,60} Studies have demonstrated that neuronal excitability is enhanced by central nervous system immune responses, which are promoted by systemic inflammation.⁶⁰ The increased neuronal excitability is induced by

abnormal hyper-synchronous electrical activity of the neuronal networks in the central nervous system^{61,62} and results in increased seizure susceptibility. Therefore, although we did not examine neuroinflammation in the current study, the higher levels of inflammatory cytokines in the PE group may result in higher seizure activity in the brain by inducing neuroinflammation. Using quantification and morphological assessment of microglia, Johnson *et al.*⁴² demonstrated that neuroinflammation was increased in severe PE. Riazi *et al.*⁶⁰ also observed increased microglial activation and number in the hippocampus of animals with peripheral inflammation. Communication between systemic inflammation and neuroinflammation involves the transport of cytokines into the central nervous system through the blood–brain barrier,⁶³ which employs neural and humoral pathways to mediate the brain response to systemic inflammation.^{64,65} Then the cytokines activate the microglia, which causes additional cytokines to be released throughout the whole brain.^{66,67}

MgSO₄ treatment decreases seizure activity and systemic inflammation in severe PE

It is well known that MgSO₄ can prevent PE from developing into eclampsia, and MgSO₄ is the drug of choice in the treatment of eclamptic seizures.^{68–71} Ohno *et al.*⁷² demonstrated that MgSO₄ decreased the risk of convulsion in eclampsia patients. In our study, we found that there was no significant difference in SBP, the levels of proteinuria, Cr, BUN, ALT and AST, the number of live fetuses and the placental weight between the P+LPS and the P+LPS+Mg groups. These results demonstrate that MgSO₄ should not be recommended to control high blood pressure during pregnancy, which is in agreement with the finding by Duley *et al.*⁷³ In addition, biomedical parameters and pregnancy outcomes have not been therapeutic targets of MgSO₄. However, MgSO₄ treatment significantly reduced both IL-1 β and TNF- α levels in the P+LPS group before and after electrical stimulation. These results indicate that MgSO₄ had anti-inflammatory effects in our PE model (P+LPS before stimulation) and eclampsia (P+LPS after stimulation). These effects were also reported by Dowling *et al.*,¹⁹ who revealed that MgSO₄ could inhibit LPS-induced inflammation and that enhanced activity of the phosphoinositide-3 kinase/Akt pathway was a crucial mechanism underlying the anti-inflammation effects of MgSO₄. Furthermore, MgSO₄ treatment increased the latency to develop stage 5 seizures, decreased the seizure duration and shortened the EEG amplitude after electrical stimulation. These results suggest that eclampsia-like seizure activity may be reduced by MgSO₄ and confirms the anticonvulsant effects of MgSO₄, which have been observed in animal studies.⁷⁴

In conclusion, LPS increases systemic inflammation in PE rats after electrical stimulation in conjunction with increases in neuronal seizure activity. The anticonvulsant, MgSO₄, decreased inflammation and seizure susceptibility. These results support the idea that increased seizure activity correlates with excessive systemic inflammation in severe PE.

Limitations of the study

First, we did not include a ‘P+MgSO₄’ control group in our study. However, Amash *et al.*⁷⁵ found that MgSO₄ does not affect maternal circulating levels of IL-1 β in the placenta during normal pregnancy but decreases circulating IL-1 β levels in the placenta during PE. These results both support and supplement our present investigation.⁷⁵ Second, our results showed that excessive systemic inflammation might have an important role in eclampsia by increasing the susceptibility to neuronal seizure activity. However, future studies are needed to clarify our present results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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