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Original Article

Rosiglitazone Prevents the Development of Kindling by Modulating Inflammatory Cytokine Production and Brain Cell Apoptosis in Mice

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Abstract

Background and objectives: Epileptogenesis involves complex mechanisms, including inflammation and apoptosis. Rosiglitazone, a peroxisome proliferator-activated receptor gamma agonist, possesses anti-inflammatory and neuroprotective properties. This study investigated whether rosiglitazone can prevent pentylenetetrazole (PTZ)-induced kindling in mice by modulating inflammatory cytokines and apoptosis pathways.

Methods: Male C57BL/6 mice (n = 8 per group) were assigned to sham, control, or rosiglitazone-treated groups. Kindling was induced with intraperitoneal PTZ (40 mg/kg) every 48 h for 17 days. Rosiglitazone (0.1 mg/kg) was administered 30 m before each PTZ injection. Seizure progression was monitored, and hippocampal tissues were analyzed via immunohistochemistry and Western blotting to assess cytokine levels (interleukin (IL)-10, IL-17A, tumor necrosis factor-alpha, interferon-gamma), caspase-3 activity, and glial fibrillary acidic protein expression.

Results: Rosiglitazone significantly delayed seizure progression, reduced seizure scores, and lowered pro-inflammatory cytokine levels (IL-17A, tumor necrosis factor-alpha, interferon-gamma) while increasing IL-10. Immunohistochemical analysis revealed fewer caspase-3-positive cells and reduced glial fibrillary acidic protein expression in the treatment group compared to controls.

Conclusions: Rosiglitazone exerts neuroprotective effects in PTZ-induced kindling, likely through its anti-inflammatory and anti-apoptotic actions. These findings underscore its potential as a therapeutic agent for mitigating epileptogenesis, warranting further investigation in combination therapies and clinical trials.

Introduction

Epilepsy is a serious and chronic neurological disorder affecting approximately 65 million people worldwide. Its primary symptom is the unpredictable onset of seizures, which can result from various pathological conditions or brain injuries, including genetic mutations and structural brain damage. Emerging evidence suggests that microglia play a significant role in epileptogenesis.[1,2] Although numerous antiepileptic drugs (AEDs) are available, they present two major limitations. Firstly, around 20–30% of patients experience refractory seizures that do not respond to AEDs. Secondly, AEDs alter excitability levels within the central nervous system, potentially resulting in cognitive impairment.[3]

Epilepsy has been linked to multiple pathogenic mechanisms, including disruptions in neuronal ion channels, alterations in gam-

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ma-aminobutyric acid and glutamate receptor functions, oxidative stress, nitric oxide pathway dysregulation, and neuroinflammation.[4] Beyond these well-established mechanisms, recent studies suggest that neurovascular dysfunction and blood-brain barrier permeability alterations contribute significantly to seizure susceptibility and chronic epileptogenesis.[5] Additionally, increased expression of several pro-inflammatory cytokines has been documented in epilepsy.[6] Clinical and experimental studies have shown elevated levels of pro-inflammatory cytokines, including interferon-gamma (IFN-γ), interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and IL-17A in epilepsy.[7,8] Consequently, targeting inflammatory pathways has emerged as a potential therapeutic strategy for managing epilepsy, highlighting the need to explore novel approaches that address inflammation-induced mechanisms underlying epileptogenesis.

In addition to neuroinflammation, oxidative stress has been identified as a critical factor in seizure pathophysiology. Experimental studies indicate that superoxide dismutase and α-tocopherol (vitamin E) play protective roles in mitigating oxidative damage in pentylenetetrazole (PTZ)-induced kindling models. This suggests that antioxidant-based therapies might complement existing AEDs by reducing neuronal damage and enhancing seizure resistance.[9]

Carbamazepine, a potent AED, has been shown to reduce inflammation by modulating prostaglandin E2-like activity and substance P concentrations.[10] Similarly, another AED, levetiracetam, exhibits anti-inflammatory properties and regulates transforming growth factor-beta 1 production.[11] Moreover, studies suggest that certain anti-inflammatory drugs, including selective and nonselective nonsteroidal anti-inflammatory drugs, possess anti-epileptic properties.[12–14] Recent research on natural compounds, such as berberine nanoparticles, has demonstrated significant anti-inflammatory, anti-apoptotic, and neuroprotective effects in PTZ-kindling models, reinforcing the central role of inflammation in seizure development.[15] These findings suggest that anti-inflammatory drugs may serve as adjuvant therapies alongside AEDs.

Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists were initially developed for diabetes mellitus.[16] These agents are known to regulate glucose and lipid metabolism, organelle differentiation, and inflammation. Recent studies suggest that PPAR-γ agonists, such as rosiglitazone, play significant roles in the nervous system,[1] exhibiting anti-inflammatory and neuroprotective effects beyond their hypoglycemic actions.[17] Moreover, emerging evidence indicates that PPAR-γ activation not only mitigates inflammation but also influences synaptic plasticity and neurotransmitter homeostasis, further supporting its potential as an anti-epileptic agent.[18] Yu *et al*. reported that the anticonvulsant activity of rosiglitazone in a lithium–pilocarpine-induced status epilepticus model was linked to its antioxidant activities,[19] while Maurois *et al*. reported that this PPAR-γ agonist was ineffective in audiogenic and ibotenate-induced seizures.[20] Nevertheless, rosiglitazone has shown promise in models of traumatic brain injury, status epilepticus, and chronic cerebral hypoperfusion.[21–24] It also exerts anti-inflammatory effects by modulating cytokines such as IL-17, IL-10, IL-6, and TNF-α.[25] Given its ability to regulate cytokine expression and oxidative stress, rosiglitazone may have therapeutic potential in preventing epileptogenesis.

The aim of this study was to evaluate the effects of rosiglitazone on PTZ-induced kindling in mice, with a focus on its anti-inflammatory and anti-apoptotic mechanisms. By investigating cytokine modulation and apoptosis pathways in the hippocampus, we sought to clarify rosiglitazone's potential as an adjuvant therapy for epilepsy.

Materials and methods

Animals

Twenty-four male C57BL/6 mice (10–12 weeks old, weighing 25–30 g) were obtained from the animal laboratory at Golestan University of Medical Sciences, Gorgan, Iran. The animals were housed in an environmentally controlled vivarium, maintained at 23 ± 2°C with a 12-h light/dark cycle and 40–50% humidity. They had ad libitum access to food and water. All experimental procedures complied with the guidelines of the Institutional Animal Care and Use Committee and were approved by the Ethics Committee of Golestan University of Medical Sciences (approval number: IR.GOUMS.REC.1394.92). Humane care was provided to all animals, and efforts were made to minimize pain and discomfort in accordance with institutional and national guidelines.

PTZ-induced model of epilepsy and experimental design

Kindling was induced through intraperitoneal (i.p.) injections of PTZ at a convulsive dose of 40 mg/kg every 48 h, administered for a total of nine subconvulsive doses over 17 days. Mice were placed in Plexiglas cages and observed for 20 m following each PTZ injection. Seizures were scored on a six-point scale, following the criteria by Ammon-Treiber *et al*.[26]

Stage 0: no response; stage 1: mouth, ear, and facial jerking; stage 2: convulsive waves axially through the body; stage 3: myoclonic jerks and rearing; stage 4: clonic-tonic convulsions, turnover into a side position; stage 5: generalized clonic-tonic seizures or lethal convulsions, loss of postural control.[27–29]

The highest observed response in each mouse was recorded as its seizure score. Mice that died from convulsions or exhibited seizures during the first three injections were excluded from the study. Kindling was considered established when a mouse reached a seizure score of 4 across three consecutive PTZ administrations.[26]

Study design

The mice were randomly allocated into three groups (n = 8 per group): 1- Sham group: Treated with 0.9% NaCl (saline, i.p.), 2- Control group: Treated with saline (0.9% NaCl) and dimethyl sulfoxide (DMSO, 1 mL/kg at 0.1% v/v, i.p.),[30] 3- Rosiglitazone-treated group: Administered rosiglitazone (0.1 mg/kg, i.p.) 30 m prior to PTZ injection (40 mg/kg).

PTZ (Sigma-P6500) was dissolved in saline, while rosiglitazone (Sigma-R2408) was prepared in DMSO and further diluted in saline at a 1:3 ratio. Each group received PTZ every other day at 10 mL/kg body weight. The selected dose of rosiglitazone (0.1 mg/kg) was based on previous studies demonstrating its efficacy in modulating neuroinflammation and apoptosis.[1,31]

Immunohistochemistry

At the end of the experiment, mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), decapitated 24 h after their last PTZ treatment, and hippocampal brain sections were collected for staining of caspase-3 and glial fibrillary acidic protein (GFAP). Brain sections were blocked in phosphate-buffered saline (PBS) containing 5% normal donkey serum, 0.02% bovine serum albumin, and 2% Triton X-100, followed by incubation at 4°C overnight in PBS with 5% normal goat serum, 0.02% bovine serum albumin, and 0.25% Triton X-100. Primary antibodies included mouse anti-GFAP monoclonal antibody [GF5] (Abcam, ab10062) and rabbit anti-caspase-3 polyclonal antibody (Abcam, ab4051). Sections were incubated overnight in a dark room at 4°C with Hoechst 33258 (Abcam, ab228550), FITC-conjugated donkey anti-mouse

IgG (Abcam, ab97029), and RRX-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch, 711-295-152). Finally, sections were rinsed three times in PBS and mounted with Aqua Poly/Mount (Polysciences Inc., Warrington, PA, USA).[32,33]

Western blotting

The hippocampus was rapidly dissected from fresh brain samples of decapitated mice, homogenized on ice, and lysed in cell lysis buffer containing 1% Triton X-100 (v/v), 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 10% glycerol (v/v), and a protease inhibitor cocktail. Total protein concentration was determined using the Bradford protein assay kit (Bio-Rad). Immunoblotting was conducted to assess levels of IL-10, IL-17A, TNF-α, IFN-γ, and β-actin. Briefly, 40 µg of protein were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred overnight onto a polyvinylidene difluoride membrane (Millipore, USA). The membrane was then blocked with nonfat dry milk, as previously described. Primary antibodies specific to IL-10 (Abcam, ab33471), IL-17A (Abcam, ab150719), TNF-α (Santa Cruz Biotech, sc-52746), IFN-γ (Abcam, ab133566), and β-actin (Abcam, ab8227) were used as a loading control. Membranes were subsequently incubated with an HRP-conjugated secondary antibody (Cell Signaling, #7076) in PBS with 0.5% dry milk and 0.1% Tween-20. Detection of target proteins was carried out using 3,3′-Diaminobenzidine with H2O2 as a substrate.[31] All protein bands were normalized to β-actin, and band intensities were quantified using ImageJ software.

Statistical analysis

Statistical analyses were performed using SPSS version 22. Multigroup comparisons were conducted using repeated-measures analysis of variance (ANOVA) and one-way ANOVA. Tukey's test was applied for post hoc comparisons. Exact p-values for all significant findings were reported to ensure clarity and precision. Statistical significance was defined as $p < 0.05$.

Results

Rosiglitazone reduced seizure severity in PTZ-induced kindling in mice

In this study, we demonstrated that repeated administration of sub-convulsive doses of PTZ induced generalized tonic-clonic seizures. Pretreatment with rosiglitazone significantly inhibited the progression of kindling compared to the control and sham groups. The stage 2 latency and stage 5 latency in the rosiglitazone group were significantly longer than those in the control and sham groups ($p < 0.05$), indicating a delay in seizure progression (Fig. 1a, b). Additionally, rosiglitazone pretreatment significantly slowed kindling progression, as evidenced by the increased interval between injections and a reduction in seizure scores ($p < 0.05$) (Fig. 1c).

Rosiglitazone modulated levels of inflammatory cytokines

The levels of IFN-γ, IL-17A, TNF-α, and IL-10 proteins were evaluated using western blotting. The results showed that rosiglitazone significantly reduced pro-inflammatory cytokines, including IFN-γ, IL-17A, and TNF-α, in the treatment group compared to the control ($p < 0.0001$) and sham ($p < 0.001$) groups. Furthermore, rosiglitazone significantly increased the levels of the anti-inflammatory cytokine IL-10 ($p < 0.0001$) compared to the other groups (Fig. 2). These findings suggest a shift in the inflammatory profile toward an anti-inflammatory state in rosiglitazone-treated mice.

Rosiglitazone reduced hippocampal neuronal apoptosis

Brain slices from the hippocampi of control and sham groups showed neuronal loss, irregular arrangement, and neurons with pyknotic nuclei in the caspase-3 region. In contrast, brain specimens from the treatment group exhibited a higher number of normally shaped neurons. The mean number of caspase-3-positive cells was significantly lower in the rosiglitazone group (14.8 ± 3.11) compared to the DMSO (23.2 ± 5.89) and saline (23.4 ± 5.68) groups ($p < 0.0001$) (Fig. 3).

Rosiglitazone reduced the number of GFAP-positive astrocytes

GFAP immunoreactivity, a widely used marker of astrogliosis, was evaluated. In hippocampal slices from rosiglitazone-treated mice, astrocyte hypertrophy in both the stratum radiatum and stratum oriens was significantly reduced. Quantitative analysis revealed a significant decrease in GFAP-positive astrocytes in the treatment group compared to the control ($p < 0.001$) and sham ($p < 0.0001$) groups, suggesting that rosiglitazone attenuates reactive gliosis (Fig. 4).

Discussion

Our study demonstrated that rosiglitazone significantly prevents the progression of kindling in PTZ-induced generalized tonic-clonic seizures in mice. Furthermore, our findings reveal that rosiglitazone reduces inflammation within the hippocampus. Microglial cells play a fundamental role in maintaining brain homeostasis, and microglial polarization is considered crucial in the pathology of various neurological disorders.[34,35] Several studies have examined microglial activation following seizures in the epileptic brain, with findings indicating that activated microglia contribute to epileptogenesis through both inflammatory and non-inflammatory processes.[19,36,37] Increasing evidence supports a strong link between seizures and neuroinflammation, as indicated by the release of pro-inflammatory cytokines from glial cells.[38] Pro-inflammatory mediators, such as TNF-α, IL-17, and IFN-γ, secreted by microglia and astrocytes, are implicated in seizure initiation. Conversely, the anti-inflammatory cytokine IL-10 has been shown to reduce febrile seizure incidence.[39,40]

PTZ-induced kindling has been associated with inflammasome activation in microglia, triggering inflammatory responses via the NF-κB pathway and the subsequent release of pro-inflammatory cytokines, including IL-18, IL-1β, and TNF-α. This inflammatory cascade can exacerbate seizures and hippocampal inflammation.[41] Interestingly, alternative therapeutic strategies, such as fenofibrate—another PPAR-γ agonist—have demonstrated significant anti-inflammatory and neuroprotective effects in epilepsy models. These findings reinforce the potential of targeting lipid metabolism pathways in seizure management, suggesting that rosiglitazone may exert additional benefits beyond cytokine modulation.[5]

Our study strengthens the understanding of these mechanisms by showing that rosiglitazone reduces hippocampal TNF-α, IL-17A, and IFN-γ levels while increasing IL-10, thereby altering the inflammatory balance toward neuroprotection. This effect is further supported by recent findings on berberine nanoparticles, which exhibit strong anti-inflammatory and anti-apoptotic properties in PTZ-kindling models. The similarity in molecular pathways affected by rosiglitazone and berberine highlights the importance of multi-targeted therapeutic strategies for epilepsy treatment.[15]

While additional experiments, such as annexin V assays and evaluations of truncated caspase-3, would provide deeper insights

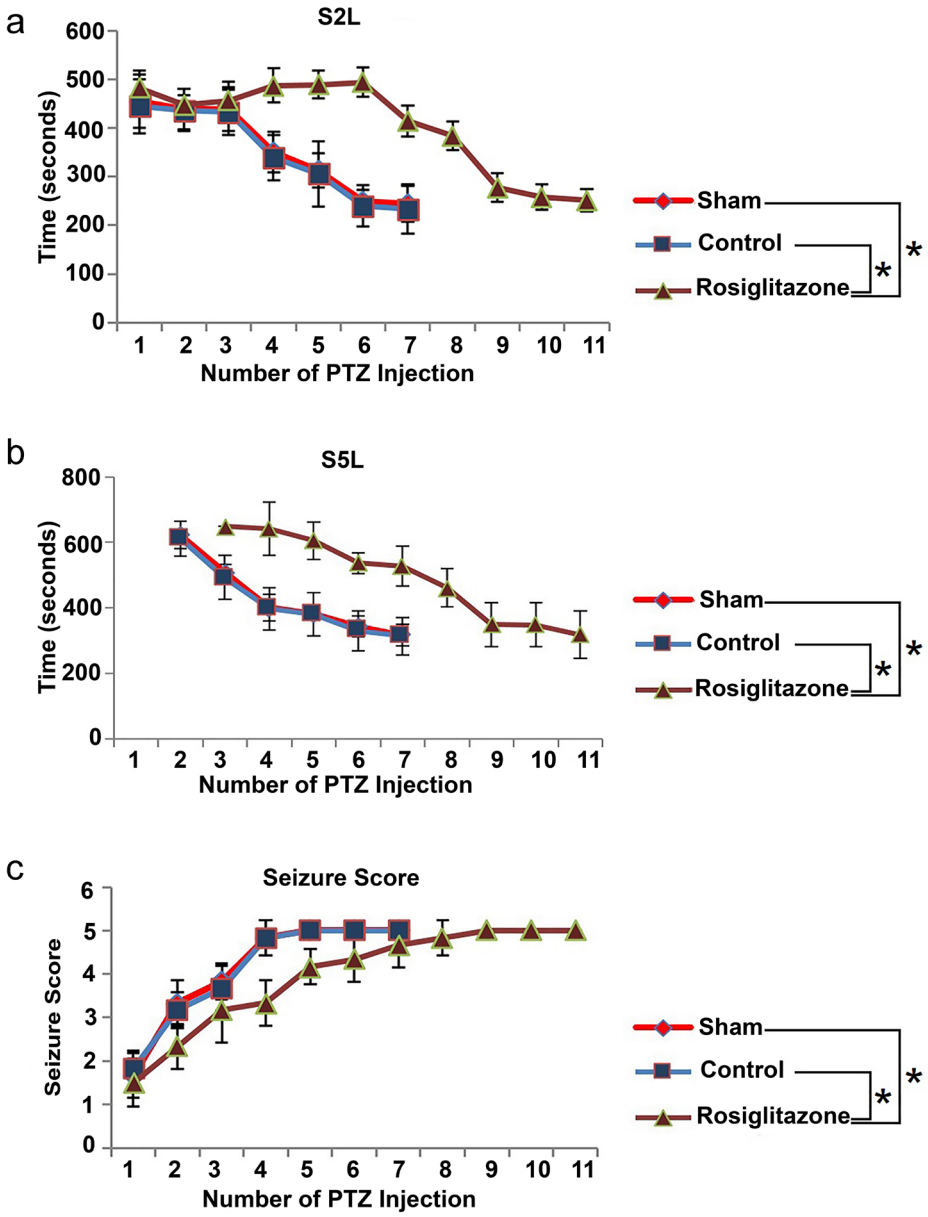

Fig. 1. Effects of rosiglitazone on seizure susceptibility in PTZ-induced kindling in mice. (a, b) The stage 2 latency (S2L) and stage 5 latency (S5L) in the rosiglitazone-treated group were significantly greater than those in the sham and control groups. (c) Statistical analysis showing a decrease in seizure scale scores following rosiglitazone treatment (two-way repeated-measures ANOVA followed by Bonferroni or Dunnett's T3 post-hoc test). Asterisks indicate significant differences between groups (*$p < 0.05$). PTZ, Pentylenetetrazole.

into apoptosis mechanisms, our current immunohistochemical analysis of caspase-3 and densitometric quantification provide a robust basis for understanding apoptosis in this model. Future research should include the evaluation of additional apoptosis- and anti-apoptosis-related proteins, along with co-localization studies using neuronal markers, to further elucidate these pathways.

Li *et al*. demonstrated that rosiglitazone reduced pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, in the hippocampus.[31] This suggests that rosiglitazone attenuates neuroinflammation, which is linked to seizure progression. Similarly, Gouveia *et al*. reported increased inflammatory cytokine levels following pilocarpine-induced status epilepticus, along with a decrease in anti-inflammatory cytokines such as IL-10.[42] Previous studies suggest that IL-10 can suppress macrophage activation, leading to a reduction in pro-inflammatory cytokines.[43,44] Our results align with these findings, suggesting that rosiglitazone enhances IL-10 expression. Notably, similar trends have been observed with embelin, a natural anti-inflammatory compound that has shown promise in preventing cognitive decline and neuroinflammation in PTZ-kindled mice. The overlap between embelin's and rosiglitazone's mechanisms further underscores the significance of cytokine modulation in epilepsy treatment.[45]

Moreover, our findings support a dual mechanism for rosiglitazone's anti-apoptotic effects, involving both direct neuroprotection

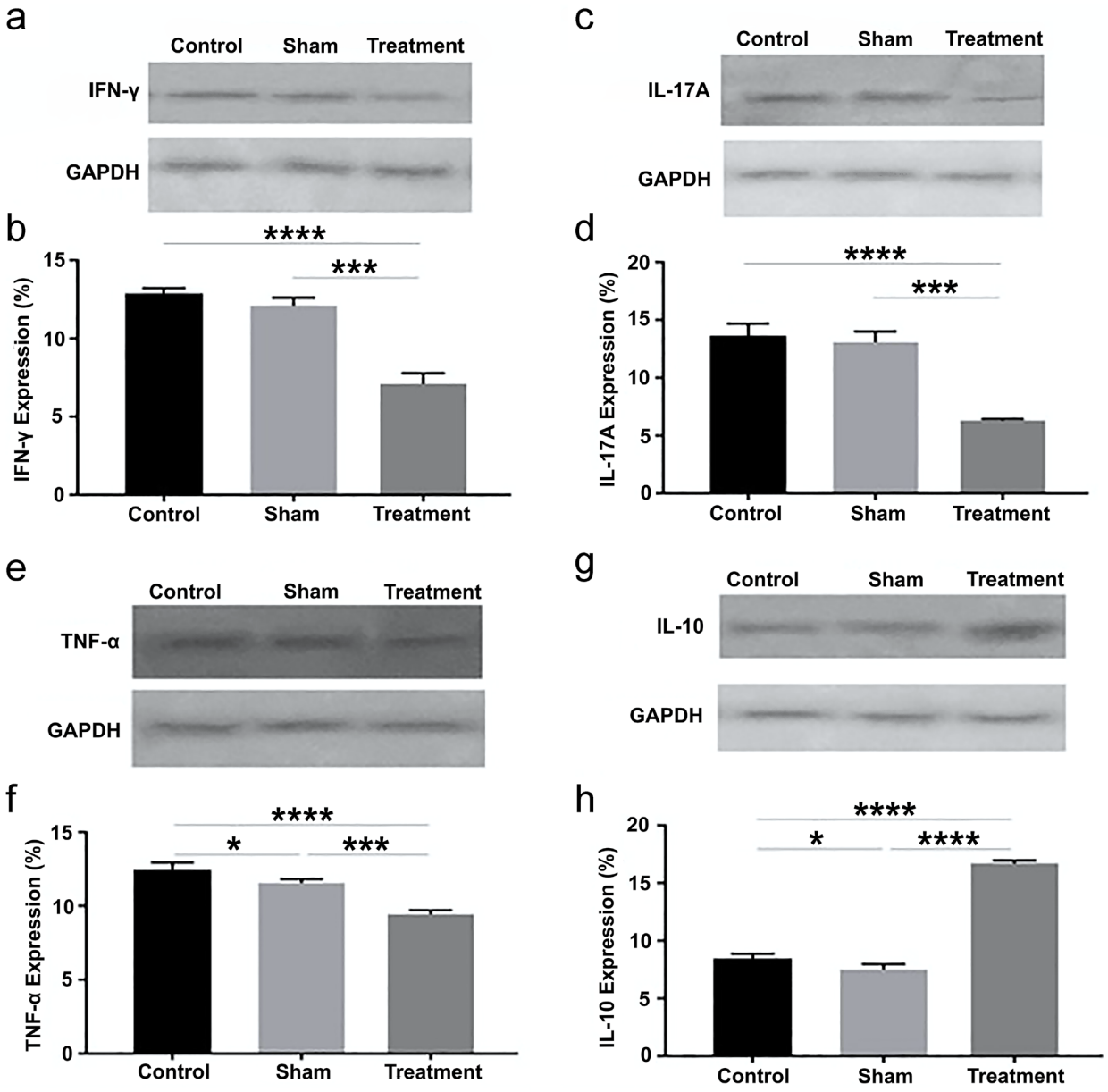

Fig. 2. Effects of rosiglitazone on the expression of inflammatory cytokines IFN-γ, IL-17A, and TNF-α, as well as the anti-inflammatory cytokine IL-10, in the hippocampus following PTZ-induced kindling. (a, c, e, g) Representative immunoblots of proteins in different groups. (b, d, f, h) Semi-quantitative analysis of IFN-γ, IL-17A, TNF-α, and IL-10 levels (n = 8/group, Student's t-test). Equal amounts of protein (20 µg) obtained from whole hippocampal homogenates were loaded per lane. GAPDH was used as an endogenous control. Error bars represent mean ± SD. Asterisks indicate significant differences between groups (*p < 0.05, ***p < 0.001, ****p < 0.0001). GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; IFN-γ, Interferon Gamma; IL-10, Interleukin 10; IL-17A, Interleukin 17A; SD, Standard Deviation; TNF-α, Tumor Necrosis Factor Alpha.

and the modulation of inflammatory cytokine secretion. Future studies employing cell-type-specific interventions and advanced imaging techniques could provide a more definitive understanding of these mechanisms.

Mao *et al*. reported an association between IL-17 and IFN-γ levels and seizure severity.[7] He *et al*. observed increased IL-17 expression in the cortex and hippocampus of patients with epilepsy, potentially contributing to neuronal damage and astrogliosis in seizure pathology.[46] Our study showed that by reducing IL-17 and IFN-γ levels, rosiglitazone exerted a protective effect against seizures. These results are consistent with findings in depression models, where rosiglitazone has been shown to modulate autophagy and inflammatory pathways to enhance neuronal resilience and function. The similarities in these mechanisms suggest that rosiglitazone's benefits may extend beyond epilepsy, potentially influencing broader neuropsychiatric disorders.[18] Recent studies also indicate that rosiglitazone's neuroprotective effects are mediated through the reduction of pro-inflammatory cytokines.[21,24]

TNF-α, a key pro-inflammatory cytokine, plays a critical role in various inflammatory processes. Beattie *et al*. demonstrated

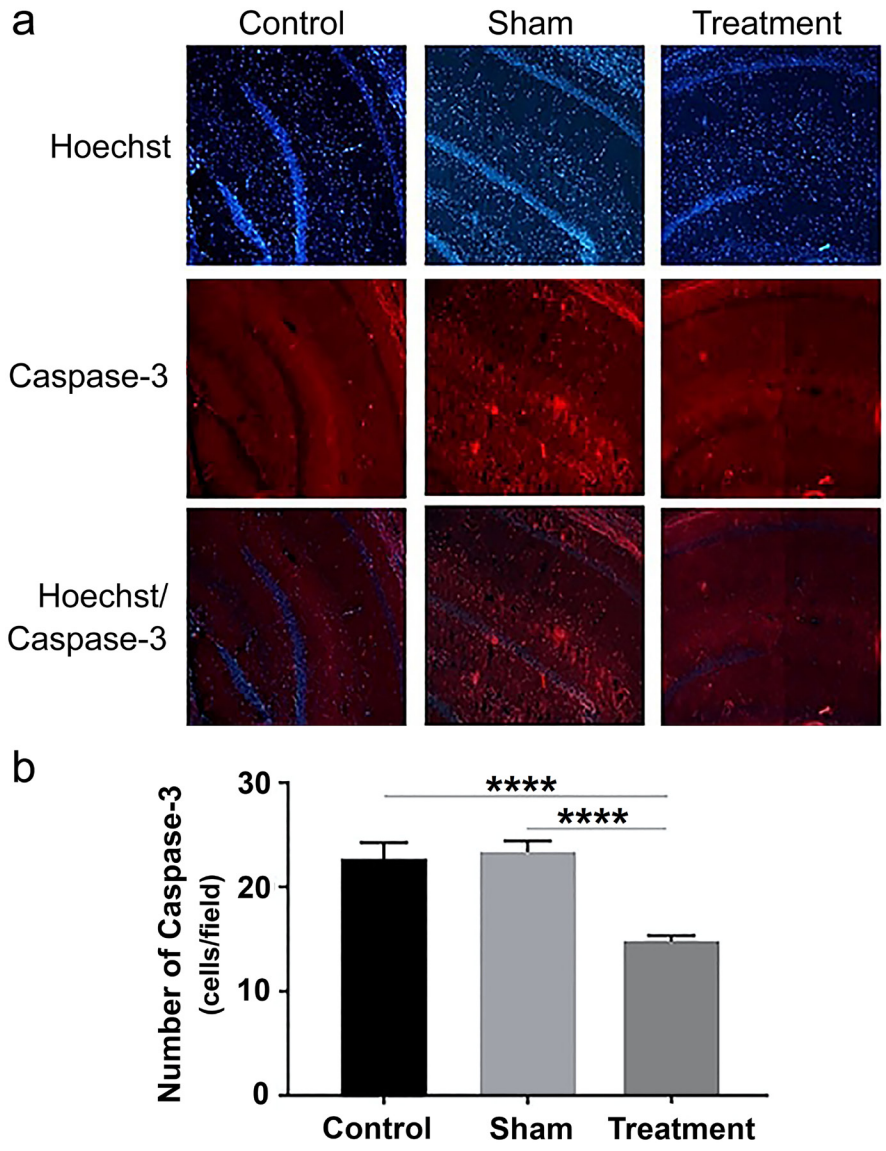

Fig. 3. Immunohistochemical (IHC) staining showing the subcellular localization of caspase-3 using an anti-caspase-3 antibody in experimental groups. (a) Representative immunofluorescence images of caspase-3 staining in the hippocampus of control, sham, and treatment groups. (b) Semi-quantitative densitometric analysis of relative caspase-3 levels in different groups. Images captured at 40× magnification. Statistical analysis was performed using one-way ANOVA followed by post-hoc testing (n = 8/group). Error bars represent mean ± SD. Asterisks indicate significant differences between groups (****p < 0.0001). SD, Standard Deviation.

that TNF-α activates α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors, increasing calcium influx and contributing to seizure initiation. Additionally, studies have shown that TNF-α influences neurotransmitter trafficking and synaptic plasticity within brain tissue.[47,48] Based on our findings, it is plausible that one of rosiglitazone's antiepileptic mechanisms involves the reduction of TNF-α levels in the brain.

Kindling has been shown to induce neuronal cell death, with previous studies reporting an increase in apoptotic cells across various brain regions following seizure induction. Rosiglitazone appears to confer neuroprotection by modulating anti-apoptotic and antioxidative pathways.[49–50] In our study, we observed an increase in apoptosis within the hippocampus following seizure induction; however, rosiglitazone administration significantly reduced apoptosis and neuronal cell death. This attenuation of apoptosis may represent a potential antiepileptic mechanism of rosiglitazone.

Astrocyte activation in response to brain injury is characterized by an upregulation of cytoplasmic intermediate filament proteins, with strong immunoreactivity for GFAP. Thus, increased GFAP expression is widely recognized as a biomarker for brain damage.[51] Studies have indicated that PTZ-induced seizures lead to an increase in GFAP-positive cells, and there is a documented association between reactive gliosis and kindling progression.[52–54] Our findings demonstrate that rosiglitazone can attenuate reactive gliosis, suggesting a potential role in slowing kindling progression.

It is important to note, however, that the long-term safety profile of rosiglitazone must be carefully considered due to its reported

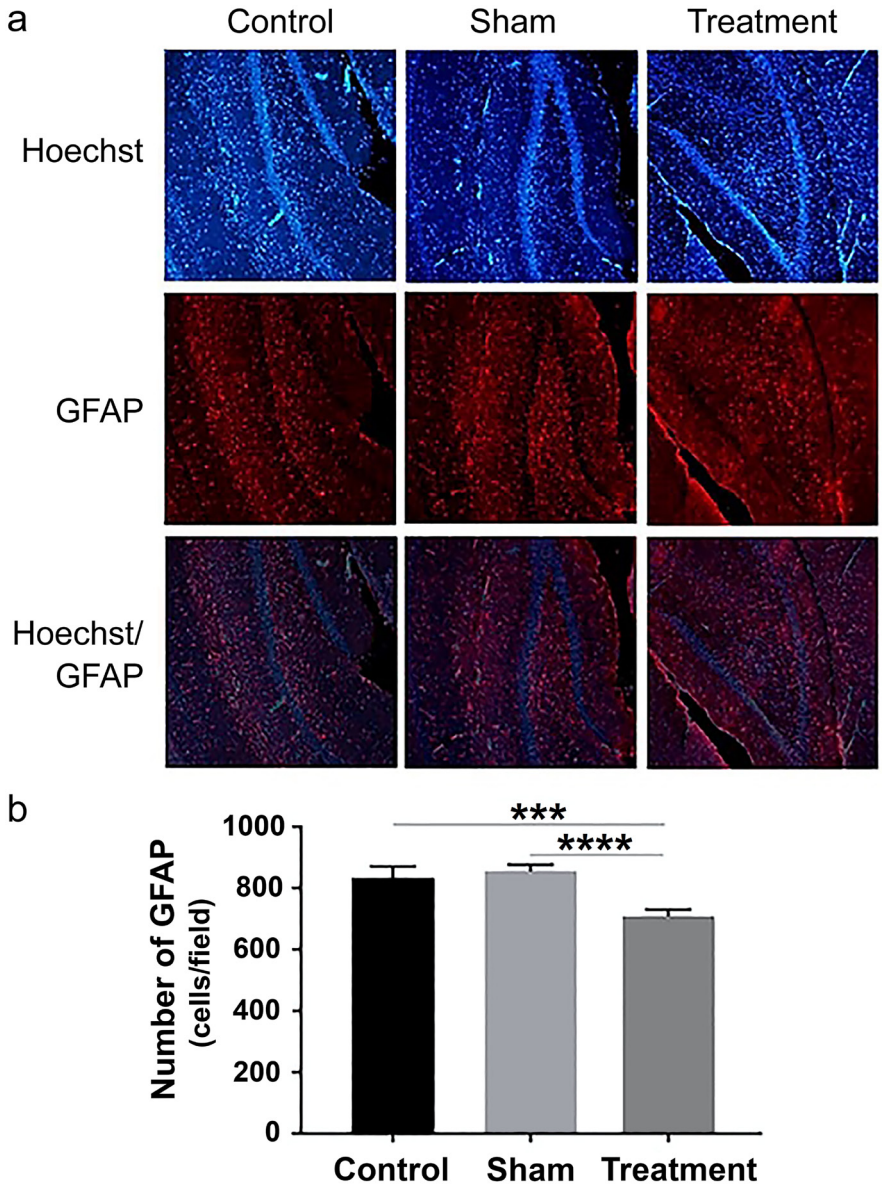

Fig. 4. Immunohistochemical (IHC) staining showing the subcellular localization of GFAP using an anti-GFAP antibody in experimental groups. (a) Representative photomicrographs of GFAP-immunoreactive astroglial cells in the hippocampus of control, sham, and treatment groups. (b) Semi-quantitative densitometric analysis of relative GFAP levels in different groups. Images captured at 40× magnification. Statistical analysis was performed using one-way ANOVA followed by post-hoc testing (n = 8/group). Error bars represent mean ± SD. Asterisks indicate significant differences between groups (***$p < 0.001$, ****$p < 0.0001$). GFAP, Glial Fibrillary Acidic Protein; SD, Standard Deviation.

association with cardiovascular disease. While these adverse effects are primarily observed in diabetic patients receiving chronic treatment, future studies are necessary to evaluate its safety in the context of epilepsy therapy.

The findings of this study open several avenues for future research. These include investigating the effects of rosiglitazone in combination with standard antiepileptic drugs, such as carbamazepine, to assess potential synergistic effects. Further studies employing advanced imaging techniques and cell-specific markers could elucidate the precise cellular targets of rosiglitazone. Additionally, exploring alternative epilepsy models, including chronic and spontaneous seizure paradigms, could provide broader insights into its therapeutic potential. Translational studies assessing rosiglitazone's efficacy and safety in human clinical trials will be essential to validate its applicability in epilepsy management.This study has several limitations. First, the sample size was relatively small, which may affect the generalizability of the findings. Second, the study was conducted in an animal model, and while mice provide valuable insights into epileptogenesis, the direct translation of these results to human epilepsy remains uncertain. Third, the study focused on a limited number of inflammatory markers and apoptotic pathways; additional molecular and cellular analyses, including oxidative stress markers and neurotransmitter alterations, would provide a more comprehensive understanding of

rosiglitazone's mechanisms. Lastly, the potential long-term side effects of rosiglitazone, particularly its impact on metabolic and cardiovascular health, were not assessed, necessitating further investigation before considering clinical applications.

Conclusions

Oxidative stress and inflammation are major contributors to brain damage and neuropathology. These processes can potentially be mitigated or treated through a combination of therapeutic strategies, including neurorestorative approaches. Our findings provide compelling evidence for the anti-inflammatory and anti-apoptotic properties of rosiglitazone, suggesting its neuroprotective effects against kindling. While our study provides a robust foundation, future research involving combination therapies, such as with carbamazepine, and additional experimental approaches will be necessary to fully elucidate the therapeutic potential of rosiglitazone. Additionally, comparative studies with emerging anti-inflammatory compounds like embelin and berberine could provide deeper insights into optimal therapeutic strategies for epilepsy management. Overall, our results indicate that the protective effects of rosiglitazone are likely mediated through the reduction of inflammation and the subsequent prevention of cell death associated with inflammatory processes.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

Author contributions

Conceived and designed this study (EH, MAZ, AA), carried out the experiments, data analysis, and results interpretation (SF, SH, MAZ, SR, AA), and performed supervision and manuscript writing (AA, EH). All authors read and approved the manuscript.

Ethical statement

All experimental procedures complied with the guidelines of the Institutional Animal Care and Use Committee and this research was approved by the Ethics Committee of Golestan University of Medical Sciences (No. IR.GOUMS.REC.1394.92). Humane care was provided to all animals, and efforts were made to minimize pain and discomfort in accordance with institutional and national guidelines.

Data sharing statement

Data associated with this study can be accessed from the authors upon reasonable request.

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