Dexmedetomidine Reduces the Lidocaine-Induced Neurotoxicity by Inhibiting Inflammasome Activation and Reducing Pyroptosis in Rats

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Local anesthetic toxicity is closely related to neuronal death and activation of the inflammatory response. Dexmedetomidine (Dex) is an adrenergic α2 receptor agonist that can reduce the neurotoxicity induced by lidocaine. It also has anti-inflammatory effects. However, the mechanism underlying the neuroprotective effects of Dex against lidocaine-induced toxicity remains to be defined. We hypothesized that Dex exerts its neural protective effect through inhibiting inflammasome activation and through anti-pyroptosis effects against local anesthetic-induced nerve injury. In a rat model of lidocaine-induced spinal cord injury, we studied the protective effect of Dex on lidocaine-induced changes in spinal cord function, inflammasome formation and pyroptosis, pro-inflammatory cytokine expression, and protein kinase C (PKC)-δ phosphorylation. Dex reduced lidocaine-induced neurotoxicity and inhibited PKC-δ phosphorylation in the spinal cord of rats. Furthermore, Dex inhibited pyroptosis and inflammasome formation (caspase-1, NLRP3, and apoptosis-associated speck-like protein (ASC)). Finally, Dex attenuated interleukin (IL)-1β and IL-18 expression, as well as microgliosis response. In conclusion, Dex can reduce the severity of lidocaine-induced spinal cord injury in rats by inhibiting priming and inflammasome activation and reducing pyroptosis via PKC-δ phosphorylation.

Key words  dexmedetomidine; pyroptosis; inflammasome; caspase 1

INTRODUCTION

Although local anesthetics are commonly used for intravertebral anesthesia, there are increasing concerns regarding their neurotoxicity. Many studies have demonstrated that the use of local anesthetics for intrathecal anesthesia can cause neurotoxicity, eventually leading to neuronal dysfunction and death.⁹ Among the commonly used local anesthetics, lidocaine has been found to produce greater neurotoxicity after spinal anesthesia.⁹ Although the mechanisms of local anesthetics-induced neurotoxicity remain to be defined, our previous study confirmed that local anesthetic toxicity is closely related to neuronal death and activation of the inflammatory response.⁵ The NLRP3 inflammasome-mediated non-apoptotic programmed cell death termed pyroptosis occurs through the activation of inflammatory caspases.⁵ Therefore, we hypothesized that pyroptosis is involved in the pathogenesis of lidocaine-induced neurotoxicity.

Dexmedetomidine (Dex) is an adrenergic α2 receptor agonist that has multiple effects, such as sedation, analgesia, anti-anxiety, and anti-convulsion. Interestingly, Dex also has protective effects against the neurotoxicity induced by local anesthetics. For example, the combination of Dex and bupivacaine can reduce the oxidative stress injury and neurotoxicity induced by bupivacaine by regulating mast cell degranulation.⁷ Due to its neuroprotective, sedative, and analgesic effects, Dex is a suitable adjuvant to local anesthetics for intrathecal anesthesia.⁸ Dex also protects the spinal cord from ischemia-reperfusion injury. For example, intraperitoneal injection of Dex can protect New Zealand rabbits from traumatic spinal cord injury by inhibiting lipid peroxidation and enhancing the activity of the endogenous antioxidant system.⁹ Intrathecal injection of Dex can protect rats from traumatic spinal cord injury through antioxidant and anti-inflammatory effects, similar to the protective effects of methylprednisolone on spinal cord injury.¹⁰ Pretreatment with Dex can also have a neuroprotective effect against ischemic injury in the spinal cord.¹¹ Specifically, Dex treatment can significantly reduce the cell structural damage of spinal cord tissue and increase the expression of the cyclic adenosine monophosphate response element binding (CREB) protein, bcl-2, and brain-derived neurotrophic factor (BDNF). This suggests that Dex can inhibit cell apoptosis and promote nerve growth through the above mechanisms. However, it is still unknown whether pyroptosis is involved in the induction of neurotoxicity by local anesthesia and whether the neuroprotective effect of Dex occurs through antagonizing the local anesthetic-induced pyroptosis. We hypothesized that Dex can inhibit inflammasome activation and alleviate the pyrotropic effects of local anesthetics, thus exerting a neuroprotective effect.

MATERIALS AND METHODS

Animals and Experimental Protocol This study included 32 male Sprague–Dawley rats (clean grade) weighing 260–300 g, aged 3–4 months. The rats were supplied by the Laboratory Animal Center of Shengjing Hospital affiliated with China Medical University. The animal protocol was approved by the Institutional Animal Ethics Committee of China Medical University (2020PS240K). All experiments were performed according to the guidelines for the ethical treatment of experimental animals. The experimental rats were randomly divided into four groups with a random number tablet (n=8) as follows: (1) sham-operation group (Sham), (2) lidocaine-induced neurotoxicity injury group (LN), (3) Dex treatment group (Dex), and (4) protein kinase C (PKC)-δ antagonist/
partial agonist,rottlerin treatment group (Rot). Dex was purchased from Jiangsu Hengrui Pharmaceutical Co., Ltd. (China). Rot was intraperitoneally injected 24 h before the operation at a dose of 0.1 mg/kg, followed by intravenous injection of Dex (1 µg/kg) via the femoral vein. In the Sham and LN groups, rats were given the same amount of physiological saline as the control for comparison.

**Intrathecal Catheterization** Intrathecal catheterization was performed as previously described. Briefly, after anesthetization with sodium pentobarbital intraperitoneally (40 mg/kg), the rats were placed in the prone position. After the L4–L5 inter-spineous space and the ligamentum flavum were exposed, a heat-connected polyethylene catheter (PE-10; Anlai Company, Ningbo, China) was inserted into the subarachnoid space at the L4–L5 intervertebral space level. A successful catheter insertion into the subarachnoid space was indicated by a tail swing or a hind leg twitch. The catheter was then further advanced caudally by 2 cm. The outflow of cerebrospinal fluid (CSF) indicated the success of the catheter insertion. After flushing with 10 µL normal saline, the distal end of the catheter was closed and fixed subcutaneously. After suturing the incision, 1 million units of penicillin (Huabei Pharmaceutical Co., Ltd., China) was administered intra-muscularly. One day after catheter insertion, 10% lidocaine (20 µL) was injected through the catheter. After lidocaine injection, the rats that became paralyzed within 30 s in both hind limbs were selected for subsequent experiments. Rats that developed unilateral limb paralysis after lidocaine injection but after catheter insertion were excluded from further experiments. For the Sham group, all rats underwent surgery for intrathecal catheter insertion and were injected with the same volume of artificial CSF (20 µL).

**Behavioral Tests** The Basso, Beattie, and Bresnahan (BBB) scale, ranging from 0 (no movement) to 21 (normal movement), was used to assess the locomotor function of the hind limbs as previously described. BBB scoring was performed on a mat four times for each rat on days 1–4. Each test was conducted for 5 min by two researchers with no knowledge of the experimental groups.

The tail-flick test was conducted in a plantar test apparatus (Ugo Basil, Italy) four times for each rat on days 1–4. Briefly, after 15 min acclimation for each rat in the testing chamber, the test was initiated by turning on the radiant heat facing the tail through the glass bottom, and the tail-flick latency (TFL) was measured using a photodiode coupled to a clock. The heat intensity was adjusted to induce a tail flick within 4–6 s in the normal control rats. The average TFL from three repeats was used for each test and the maximal heating time was 20 s to avoid tissue damage.

**Terminal Deoxynucleotidyl Transferase-Mediated Deoxyuridine Triphosphate Nick-End Labeling (TUNEL) Staining** Paraffin-embedded spinal cord tissue sections were prepared for TUNEL staining. After deparaffinization and rehydration, the tissue sections were treated with 3% H₂O₂ for 10 min at room temperature, then digested with proteinase K with 1:200 dilution in Tris-buffered saline (TBS) for 30 min at 37 °C. The tissue sections were treated with 0.01% Triton-X100 for 20 min at 25 °C. The in situ Cell Death Detection Kit (Cat. No. 11684817910, Roche, Germany) was used for TUNEL staining. Briefly, tissue sections were first incubated with terminal deoxynucleotidyl transferase (TdT) and deoxyuridine triphosphate (dUTP)-digoxigenin at 37 °C for 2 h. After three washes with TBS, the tissue sections were stained with 3,3’-Diaminobenzidine (DAB) and hematoxylin. Four sections from each rat were used. Five fields from each section in the injured area were randomly selected for examination under a light microscope with 400× magnification. The images were analyzed with the Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, U.S.A.). The percentage of TUNEL positive cells out of the total number of cells was calculated for the final presentation.

**Immunohistochemistry** The tissues were post-fixed and dehydrated in 30% sucrose in phosphate-buffered saline (PBS) at 4 °C for 24 h and frozen sections were prepared. The spinal cord tissue sections (10 µm thick) were incubated at 4 °C overnight with a rabbit anti-α rat primary antibody against ionized calcium-binding adapter molecule 1 (Iba1, 1:200 dilution, ab5076; Abcam, U.S.A.), followed by incubation with biotinylated anti-rabbit immunoglobulin G (IgG) antibodies (1:200 dilution; Vector Laboratories, Burlingame, CA, U.S.A.) in 1.5% normal donkey serum (NDS; Jackson Immunoresearch Laboratories Inc., West Grove, PA, U.S.A.) for 20 min at 37 °C. All sections were covered-slipped with a mixture of 50% glycerin in 0.01 mol/L PBS and then observed under a Leica SP2 confocal laser scanning microscope (Leica, Wetzlar, Germany). The average optical density (AOD) of immunoreactive staining for Iba1 was measured with the Image J analysis system (National Institutes of Health, Bethesda, MD, U.S.A.). For each animal, eight sections of spinal cord in the L4–L6 level were randomly selected for quantitative evaluation. The corrected density values of the eight sections were averaged to provide a mean density for each animal. All behavioral testing and the quantification of immunohistochemical experiments were performed blind with respect to treatment.

**Western Blot Analysis** The expression of caspase-1, NLRP3, and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) in the spinal cord was determined using Western blot analysis. Cells from the spinal cord tissue were lysed using radioimmunoprecipitation assay (RIPA) lysis buffer (Shanghai Beyotime Biological Technology Co., Ltd., China). After determining the protein concentration, an equal amount of protein for each sample was used to load sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). The proteins were separated by electrophoresis and transferred onto positively charged nylon membranes, which were then incubated with the following primary antibodies: rabbit-anti-rat caspase-1 antibody (1:1000, Santa Cruz Company, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, No. AF4022, Affinity, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, No. ab179515, Abcam, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, #50928, Cell Signaling Technology Company, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, #96458, Cell Signaling Technology Company, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, #50928, Cell Signaling Technology Company, U.S.A.), rabbit-anti-rat caspase-1 antibody (1:1000 Dilution, #96458, Cell Signaling Technology Company, U.S.A.) for both the pro and cleaved forms, 1:1000 Dilution, Cell Signaling Technology Company) and IL-18 (for both the pro and cleaved forms, 1:1000 Dilution, Cell Signaling Technology Company) antibodies, and rabbit-anti-rat NLRP3 antibody (1:1000, Santa Cruz Company) at 4°C overnight. The membranes were
washed with TBS the next day and blotted with horseradish peroxidase (HRP)-labeled IgG goat-anti-rabbit secondary antibody (1:1000, Santa Cruz Company) at room temperature for 1 h, followed by visualization using an enhanced chemiluminescence (ECL) reagent and a gel imaging analysis system. Caspase-1, NLRP3, and ASC expression levels were determined by comparing the grayscale values of the protein bands to that of the internal reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using Scion Image software. The ratio of a target protein to GAPDH was used as the relative expression of the target protein.

RT-PCR Total RNA was purified from the spinal cord with Trizol reagent (Invitrogen, U.S.A.). The concentration of the extracted RNA was measured using a nucleic acid quantitation instrument (Thermo Company, U.S.A.). One microgram total RNA was used for each reverse transcription (10 µL system). RT-PCR was performed with the TaKaRa RNA PCR Kit Ver3.0 (Shanghai Sangon Biotechnology Co., Ltd., China) using the primers listed in Table 1. We used Scion Image software to analyze the grayscale values of the PCR bands for each target and normalized these to the internal reference, β-actin, to estimate the IL-1β and IL-18 mRNA levels.

**Statistical Analysis**
The statistical analyses were performed with the SPSS 21.0 software. The data were tested to confirm the normal distribution and are expressed as mean ± standard deviation. Differences between groups were compared using one-way ANOVA. When statistically significant, the Student–Newman–Keuls method was used for pairwise comparison. *p* < 0.05 was considered statistically significant.

**Table 1. Primers for RT-PCR**

| Primer          | Sequence (5’–3’ sense)       |
|-----------------|-------------------------------|
| IL-1β forward   | 5’-CTCAACTGTGAAATAGCAGCTTTTC-3’ |
| IL-1β reverse   | 5’-GGGACGGCCTAAATGCTTGCTG-3’  |
| IL-18 forward   | 5’-ATGTCAGCCGCAAGACGAC-3’     |
| IL-18 reverse   | 5’-TTCATCCTCAGTAGCTGG-3’      |
| β-Actin forward | 5’-GGGAGATATGCGCTGGTCTCTA-3’  |
| β-Actin reverse | 5’-GACTCATGTACCTCTGCTG-3’     |

**RESULTS**

**Dex Reduces Lidocaine-Induced Neurotoxicity in the Spinal Cord**
The effect of Dex on lidocaine-induced neurotoxicity in the spinal cord was investigated using tail-flick tests and the standard neurological disability scoring. As shown in Fig. 1A, the hind limb locomotor function decreased significantly 2 d after lidocaine treatment, evidenced by a reduction in the BBB score. This effect lasted until the fourth day with little recovery when compared to the sham control (*p* < 0.05), further confirming the neurotoxicity of the lidocaine injection. We then tested whether Dex pretreatment had a protective effect on the lidocaine-induced neurological disability. Indeed, as shown in Fig. 1A, Dex pretreatment significantly improved the BBB score of the lidocaine-treated rats (*p* < 0.05) on day 2 and facilitated recovery on days 3 and 4. To further explore the molecular mechanism, we hypothesized that PKC-δ was involved in the neuroprotective effect of Dex. As shown in the figure, pretreatment with the PKC-δ antagonist/partial agonist Rot abolished the beneficial effects of Dex. The tail flick latency was also measured 3 d after lidocaine administration to further evaluate the neurotoxicity of lidocaine. As shown in Fig. 1B, lidocaine treatment significantly increased the tail flick latency when compared with that in the Sham control (*p* < 0.05). Again, pretreatment with Dex significantly reduced the magnitude of the lidocaine-induced increase in latency (*p* < 0.05 compared with the LN group). Similarly, pretreatment with Rot abolished the beneficial effects of Dex on tail flick latency, providing another line of evidence to support the involvement of PKC-δ in the Dex-mediated neuroprotection. Thus, systemic administration of Dex can have a neuroprotective effect against intrathecally administered lidocaine-induced spinal cord damage and this protective effect is likely related to PKC-δ.

**Dex Inhibits Lidocaine-Induced Neural Pyroptosis in the Spinal Cord** To investigate the effect of Dex on neuronal pyroptosis caused by lidocaine-induced neurotoxicity in the spinal cord, we compared the percentage of TUNEL positive cells in each group. Lidocaine administration significantly increased the percentage of TUNEL positive cells in the spinal cord when compared to that in the Sham control, implying

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**Fig. 1. Dex Reduces Lidocaine-Induced Neurotoxicity in the Spinal Cord**

(A) The hind limb locomotor function was evaluated based on the BBB scores for 4 consecutive days after surgery for the groups as indicated. Dex treatment significantly reduced lidocaine-induced spinal cord injury and facilitated recovery, as indicated by higher BBB scores. The protective effect of DEX on disability was abolished by co-treatment with the PKC-δ antagonist Rot. *N* = 8. (B) Dex treatment significantly reduced the lidocaine-induced prolongation of tail flick latency and facilitated recovery. Rot abolished the protective effect by Dex. *: *p* < 0.05 compared to Sham; **: *p* < 0.01 compared to Sham; *p* < 0.05 compared to LN or Rot. *N* = 8.
an increase in neural pyroptosis after lidocaine-induced neurotoxicity. Dex pretreatment significantly reduced the lidocaine-induced increase in the percentage of TUNEL positive cells in the spinal cord. The protective effect of Dex against lidocaine-induced pyroptosis was abolished by Rot treatment (Figs. 2A, B). Since TUNEL positive staining can reflect both pyroptosis and apoptosis, we further performed Western blot analysis for the pyroptosis-specific gasdermin D activation to differentiate between these two conditions. As shown in Figs. 2C, D, the gasdermin D P30 fragment (MW: 30 kD) in the spinal cord increased by approx. 10 fold after lidocaine treatment. This further suggests that lidocaine induced pyroptosis in our spinal injury model. Furthermore, the lidocaine-induced increase of the P30 fragment of gasdermin D could be significantly reduced by Dex pretreatment, and this beneficial effect was abolished by treatment with the PKC-δ antagonist/partial agonist Rot. Rot is generally regarded as a PKC-δ kinase inhibitor. However, it has been reported that it is also a weak agonist for PKC-δ. The data in Figs. 3A and B show that Rot treatment partially restores the p-PKC-δ level. This is potentially due to its agonist effect. Regardless of the mechanism, the effect of Rot on the p-PKC-δ level was in parallel with its effect in the functional test, suggesting that the inhibition of PKC-δ by Dex is likely one of the mechanisms for its neuroprotective effect.

Dex Inhibits Inflammasome Formation To explore the potential role of Dex in suppressing inflammasome formation, we measured caspase-1, ASC, and NLRP3 expression in the spinal cord of experimental rats in each group. Intrathecal administration of lidocaine significantly enhanced caspase-1 (active form), ASC, and NLRP3 expression in the spinal cord when compared to that in the Sham control, indicating the formation of inflammasomes in spinal cord cells after lidocaine-induced neurotoxicity. However, Dex pretreatment significantly reduced lidocaine’s effects on the expression of
these inflammasome factors, suggesting that pretreatment with 1 µg/kg Dex inhibits neural pyroptosis through inhibiting inflammasome formation (Fig. 4).

Dex Attenuates the Inflammatory Response To investigate the effect of Dex on the inflammatory response initiated by lidocaine-induced neurotoxicity, we examined the expression of important inflammatory mediators for pyroptosis including IL-1β and IL-18 in the spinal cord. Lido
caine treatment significantly increased the mRNA expression levels of IL-1β and IL-18 when compared to the levels in the Sham group. Combined with the above results, this effect is likely due to the activation of caspase-1, which can promote the expression of the pro-inflammatory cytokines IL-1β and IL-18. However, pretreatment with Dex effectively inhibited the lidocaine-induced upregulation of these cytokines, which was reversed by Rot treatment (Fig. 5A). In order to determine whether cleaved and activated forms of IL-1β, IL-18, pro IL-1β and pro IL-18 also had similar changes, we performed Western blot analysis for IL-1β, IL-18, pro IL-1β and pro IL-18. The pro and active forms of these two cytokines also exhibited changes similar to those in their mRNA levels (Figs. 5B–E). These results demonstrated that lidocaine also has a priming effect that increases the gene expression of pro-
inflammatory cytokines and that Dex pretreatment can signifi-
cantly inhibit both the priming step and the downstream in-
flammatory response of pyroptosis, which is mediated through

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Fig. 3. Dex Inhibits PKC-δ Phosphorylation
(A) Western blot analysis of total and phosphorylated PKC-δ in the spinal cord of four groups of rats. (B) Quantitative analysis of the Western blot images of band intensities and related p-PKC-δ expression normalized to the total PKC-δ.

Fig. 4. Dex Inhibits Inflammasome Formation
(A) Western blot analysis of the expression of the important inflammasome factors, caspase-1, NLPR3, and ASC, in the spinal cord of four experimental groups; (B) Quantitative analysis of the Western blot results in (A) normalized to the internal control GAPDH. *p < 0.05, N = 4. (C) Western blot analysis of the uncleaved form of caspase-1. (D) Quantitative analysis of the cleaved forms of caspase-1 normalized to the uncleaved forms. N = 4.
PKC-δ. Since Dex treatment also significantly influences the pro forms of IL-1β and IL-18 at the protein level, it is likely that Dex exerts its effects in both the priming and activation steps. In addition, microglia play the role of macrophages and participate in the immune inflammatory response of the central nervous system. Figures S5 and G show that the AOD of microglia in the LN group was enhanced compared to that in the Sham group, suggesting enhanced neuroinflammation after LN treatment. However, with Dex treatment, the AOD of microglia was significantly decreased compared to that in the LN group, providing another line of evidence for the anti-inflammatory effect of Dex.

**DISCUSSION**

Lidocaine-induced neurotoxicity involves inflammatory responses. Dex has a neuroprotective effect against the neurotoxicity induced by lidocaine as well as an anti-inflammatory effect. However, the detailed mechanism for the neuroprotective effect of Dex against lidocaine-induced toxicity remains to be defined. In this study, we uncovered several lines of evidence suggesting that the neuroprotective effect of Dex on lidocaine-induced spinal cord injury occurs through its anti-inflammamson and anti-pyroptosis effects in both the priming and activation steps.

Pyroptosis is a unique programmed cell death distinct from apoptosis and necrosis. Unlike apoptosis, it depends on the activation of caspase-1 rather than caspase-3. Pyroptosis is characterized by NLRP3 inflammasome activation, maturity, release of caspase-1 and its downstream cytokines, and subsequent non-cellular inflammation and apoptosis. Recently, more studies have focused on the phenomenon of neurotoxicity caused by anesthesia-mediated neuronal pyroptosis. For example, for ketamine-induced hippocampal neurotoxicity, caspase-1-dependent pyroptosis is an essential pathway for apoptosis. Pyroptosis and apoptosis are involved in the neurotoxicity of sevoflurane, which can be alleviated by erythropoietin via the extracellular signal-regulated kinase 1/2—nuclear factor-E2-related factor 2 (Erk1/2–Nrf2) Bach1 signal pathway. In this study, we found that pyroptosis is also involved in the mechanism of lidocaine-induced neurotoxicity in damaged spinal cord tissue, suggesting that the pyroptosis pathway is a promising novel target for the prevention and treatment of local anesthetic-induced nerve injury.

Caspase-1 is an enzyme that converts the precursors of IL-1β and IL-18 into active inflammatory cytokines, which eventually mediate cell death. Caspase-1 also cleaves the gasdermin D protein, resulting in gasdermin D pore formation in the plasma membrane. The pro-inflammatory cytokines are subsequently released through the membrane pore, contributing to pyroptosis. Thus, caspase-1 activation and maturation of the pro-inflammatory factors IL-1β and IL-18 are important mechanisms for pyroptosis. The inflammasome plays a key role in innate immunity. It is a multiprotein complex that mediates the production and release of the pro-inflammatory cytokines IL-1β and IL-18. Nod-like receptor is a cytoplasmic pattern recognition receptor, and some of its subgroup members, such as NLRP3, are involved in inflammasome formation. Dysregulation of IL-1β is associated with immune pathology, and the inhibition of PKC-δ can reduce p65 phosphorylation and pro-IL-1β production in T cells. The PKC-δ signaling pathway also regulates IL-18-induced matrix metalloproteinase (MMP)-13 expression. In our study, we found that the regulation of PKC-δ phosphorylation affects inflammasomes and cell pyroptosis. All of our findings suggest that PKC-δ phosphorylation mediates the occurrence of focal cell death in the spinal cord of local anesthetic-induced neurotoxic rats.

Dex also protects the brain against inflammation. In lipopolysaccharide-induced major glial cell injury in the brain, Dex plays a neuroprotective role, mainly by reducing the production of IL-1 and tumor necrosis factor (TNF)-α and inhibits the inflammatory response at high doses. Its anti-inflammatory effect is achieved by blocking the nuclear translocation and binding of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and reducing the level
of IL-6 and TNF in rats.\cite{32} After Dex inhibits the NF-κB pathway, the expression of Bax and caspase-1, which are important target genes for NF-κB to regulate apoptosis, is inhibited. Bax is involved in apoptosis and migrates from the cytosol to the mitochondria to degrade the nuclear membrane, promoting apoptosis.\cite{33} Caspase-1 exists in cells, and after receiving pro-apoptotic signals, it regulates the effect of other caspases, which are the executive proteins for apoptosis.\cite{34} In this study, we found that Dex can reduce the expression of inflammatory cytokines in the spinal cord in lidocaine-induced neurotoxic rats. This is consistent with previous findings.

PKC has many isoforms, some of which are highly expressed in the central nervous system. There are three structurally and functionally different subfamilies of PKC isoforms: conventional isoforms (α, βI, βII, and γ), novel isoforms (ε, δ, η, and θ), and atypical (ζ and iζ) isoforms.\cite{35} The conventional isoforms are sensitive to calcium, whereas the novel and atypical isoforms are insensitive to calcium. The conventional isoforms are expressed in the dorsal horn of the spinal cord, contributing to neuronal plasticity.\cite{36} PKC activation can regulate neuronal excitability through modulating neurotransmitters and their receptors in the spinal cord.\cite{37} Dex can also have a neuroprotective effect against lidocaine-induced neurotoxicity in the spinal cord through modifying PKC-βI expression and glutamate release.\cite{39} In our study, we found that the PKC-δ signaling pathway is the key pathway through which Dex exerts its anti-inflammatory effects. Regulating inflammatory reactions has become a key strategy for the treatment of nervous injury. We have demonstrated that the anti-inflammatory effect of Dex is an important mechanism for its neuroprotective effect.

The proper dose of Dex to achieve a neuroprotective effect is also important. For example, 50 μg/kg Dex has protective effects on the hippocampus neurons, whereas a 500 μg/kg Dex can result in neurotoxicity, causing the impairment of cognitive function in rats.\cite{40} In our study, the dosage of Dex was selected in accordance with the drug instructions to ensure safety and take into account clinical practicality. A previous study showed that the administration of a high dose of Dex may have more pronounced effects,\cite{40} but doses beyond the suggested limit cannot be applied in clinical practice. Thus, we selected the recommended dosage of Dex. We expect that the data in this study will be helpful for future clinical practice and ultimately benefit patient care.

Finally, the mechanism of lidocaine neurotoxicity is not completely clear. Although we have provided several lines of evidence that it can induce pyroptosis, it remains to be further confirmed by caspase-1 knockout in the future study. In conclusion, Dex pretreatment can reduce the severity of lidocaine-induced neurotoxicity injury in rats by inhibiting priming and inflammasome activation and reducing pyroptosis via PKC-δ phosphorylation.

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Conflict of Interest The authors declare no conflict of interest.

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