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Pretreatment with resveratrol ameliorate trigeminal neuralgia by suppressing matrix metalloproteinase-9/2 in trigeminal ganglion

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ABSTRACT

Trigeminal neuralgia (TN) is a common type of neuropathic pain whereas the underlying pathogenesis has not been completely elucidated. Recent study suggests that the development of neuroinflammation is responsible for generating and sustaining neuropathic pain. The purpose of our study was to investigate the protective effect of intervening the inflammation in early stages of pain and explore its potential mechanism. MMP-9 and MMP-2 are vital proinflammatory participants and accumulating evidence indicates that they are involved in the early development of neuropathic pain. In this study, we found that MMP-9/2 showed different temporal up regulation in trigeminal ganglion (TG) significantly after chronic constriction injury (CCI) surgery. However, the activation of MMP-9/2 were suppressed by the pretreatment with resveratrol, which delayed and attenuated CCI-induced mechanical allodynia simultaneously. Besides, the expression of proinflammatory cytokines like IL-1β and TNF-α as well as the excessive neuronal activity induced by CCI were suppressed by resveratrol. Moreover, we believed that the inhibition of MMP-9/2 activation and pain sensitization may be related to the TLR-4/NF-κB signaling pathway, which might be negatively regulated by the induction of SOCS3. In conclusion, pretreatment with resveratrol could be an effective approach to alleviate trigeminal neuralgia in early stages via a powerful inhibition on the activation of MMP-9/2 in TG.

1. Introduction

Trigeminal neuralgia (TN) is a disabling condition and the impact of which on the lives of patients is profound [1]. Nevertheless, the underlying pathogenesis of TN has not been completely elucidated. As a common type of neuropathic pain, TN is generally understood to result from alterant neuronal plasticity [2]. Recent progress suggests that the development of neuroinflammation is responsible for generating and sustaining neuropathic pain as well [3–5]. Neuroinflammation refers to a localized inflammation occurring in the nervous system, which can be triggered by multiple insults like trauma or infection. Given its capacity for sensitizing nociceptive neurons that leads to neuropathic pain [6,7], targeting the processes and molecules that are involved in neuroinflammation could lead to better treatments for TN.

Accumulating evidence indicates that matrix metalloproteases (MMPs) are widely implicated in the development of neuropathic pain [6,8,9]. They are a large family of zinc-dependent endopeptidases which are critical in pain induction and sensitization [8,10]. Among all the family members, MMP-9 and MMP-2 are most studied which could cleave the extracellular matrix proteins, cytokines, chemokines and are widely implicated in tissue remodeling [11]. Consistently, after peripheral nerve injury, a rapid up regulation of MMP-9 was observed in dorsal root ganglion (DRG) in early stages of pain [8]. Inhibiting MMP-9/2 activity could prevent interleukin (IL)-1β cleavage and contribute to the attenuation of peripheral inflammation-induced thermal and tactile hypersensitivity [9,12]. Thus, we further hypothesize that MMP-9 and MMP-2 may take part in the peripheral neuroinflammation in TN.

Notably, increasing evidence reveals that MMP-9/2 could be

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Abbreviations

TN	trigeminal neuralgia
MMP-9/2	matrix metalloproteinase-9/2
TG	trigeminal ganglion
CCI	chronic constriction injury
TLR-4	Toll-like receptor-4
SOCS3	suppressor of cytokine signaling 3
DRG	dorsal root ganglion
STN	spinal trigeminal nucleus
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
NR1	NMDA receptor 1
PKCγ	protein kinase C γ
AMPK	5′-monophosphate (AMP)-activated protein kinase

upregulated by the activation of Toll-like receptor(TLR)-4 signaling [13–15], which is a critical signaling node for the induction of inflammation [16]. Inhibiting TLR-4 signaling effectively suppressed MMP-9 activity in LPS-induced airway inflammation [17]. Our previous research indicated that intraperitoneal administration of a TLR-4 antagonist in mice significantly inhibited nerve injury-induced neuroinflammation [18]. On account of that TLR-4 inhibitors currently applied in experimental research have shown certain liver toxicity and are clinically unavailable [19], we pay attention to an endogenous anti-inflammatory protein called suppressor of cytokine signaling (SOCS)3. SOCS3 belongs to a family of intracellular negative regulatory proteins and is identified as a key role at the cross roads of numerous pathological events [20,21]. Previously we found that lentiviral-mediated SOCS3 over-expression could substantially ameliorate postoperative pain via inhibiting TLR-4 signaling pathway [18]. Thus, we speculate that SOCS3 may negatively regulate TLR-4 signaling pathway to decrease the expression of MMP-9/2.

In TN mice model, neuroinflammation in spinal trigeminal nucleus (STN) and hyperalgesia could be relieved by resveratrol (trans-3, 5, 4′-trihydroioxystilbene, Res) in our previous study [22]. Resveratrol is a natural polyphenolic compound which is widely employed in anti-aging effect as well as cardiovascular protection due to its oxidation resistance and anti-inflammatory properties [23]. Recently, resveratrol has been reported to be able to ameliorate neuropathic pain [24], whereas the detailed mechanisms have not been fully elucidated. Meanwhile, traditional pharmacological interventions were mostly taken after pain eventually formation and sufferers have been subjected to torture for a long time.

In this study, we provide the first evidences that pretreatment with resveratrol, a safe and effective natural product, could delay and ameliorate CCI-induced neuropathic pain in early stages via the suppression of MMP-9/2 in trigeminal ganglion (TG). We further hypothesize that this inhibitory effect is associated with the endogenous “immune brakes” SOCS3 and its negative regulation to TLR-4 signaling pathway.

2. Materials and methods

2.1. Ethics statement

All procedures were strictly performed in accordance with the regulations of the ethics committee of the International Association for the Study of Pain and the Guide for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of China, 2006). All animal experiments were approved by the Nanjing Medical University Animal Care and Use Committee and designed to minimize suffering and the number of animals used.

2.2. Animals

Adult CD-1 mice (18–22 g) were provided by the Experimental Animal Center at Nanjing Medical University, Nanjing, China. Animals were housed five to six per cage under pathogen-free conditions with soft bedding under controlled temperature (22 ± 2 °C) and a 12 h light/dark cycle (lights on at 8:00 a.m.). Behavioral testing was performed during the light cycle (between 9:00 a.m. and 5:00 p.m.). Animals were allowed to acclimate to these conditions for at least 2 days before starting experiments. For each group of experiments, the animals were matched by age and body weight. At the end of experiments, all mice underwent euthanasia with injection of overdose sodium pentobarbital (4%, 50 mg/kg, i.p.).

2.3. Surgery

The TN model was produced by chronic constriction injury to the unilateral infraorbital nerve (CCI-IoN) via an intraoral approach as described previously [25,26]. Animals were anesthetized with sodium pentobarbital (4%, 50 mg/kg, i.p.) and all surgeries were performed aseptically. The head was fixed and the mouth kept open during the operation. Ophthalmic cream was applied to the corner of both eyes to prevent drying damage. A 1-cm incision was made along the left gingivobuccal margin in the buccal mucosa, beginning immediately next to the first molar. The left infraorbital nerve was freed and loosely tied with 2 to 4 chromic gut (4–0) ligatures 1.5–2 mm apart, after which the incision was closed. Sham-operated animals received only nerve exposure but no ligation.

2.4. Von Frey test

Mechanical sensitivity of the whisker pad, the infraorbital nerve receptive field, was measured with a series of Von Frey fiber filament (Stoelting, Wood Dale, IL). Before tests, each mouse was shaved around the whisker pad and handled for 3–5 days (once a day for 30 min). One experimenter gently held the mouse in an insulating cotton glove until the animal was calm. A series of calibrated Von Frey filaments ranging from 0.07 to 4 g were lightly applied the whisker pad, both ipsilateral and contralateral to the surgery site. A brisk or active withdrawal of the head from the probing filament was defined as a response. Each filament was tested 5 times at 5-s intervals. The withdrawal threshold was defined as the lowest force in grams that produced at least 3 withdrawal responses in 5 consecutive applications [26,27]. All behavioral tests were conducted under blind conditions.

2.5. Drugs and reagents

Resveratrol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cli-095, SB-3CT were purchased from MedChemExpress (Monmouth Junction, NJ). Antibodies for TNF-α, phospho-NF-κB p65 (Ser536) phosphorylated NR1 subunit (Ser896), phosphorylated protein kinase C (PKC) (pan)(gamma Thr514) were from Cell Signaling Technology (Beverly, MA, USA). Antibodies for IL-1β were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody for β-actin was from Sigma-Aldrich (St. Louis, MO, USA). Antibody for SOCS3 was from Abcam (Cambridge, MA, USA). Secondary antibodies were from Cell Signaling Technology (Beverly, MA,USA). All other reagents were from Sigma-Aldrich (St. Louis, MO, USA).

2.6. Gelatin zymography

Animals were anesthetized deeply with sodium pentobarbital (4%,

50 mg/kg, i.p.) and TGs ipsilaterally to the surgery site were rapidly dissected and homogenized in 1% NP40 lysis. 300 to 500 μg of protein per lane was loaded into the wells of precast gels (8% polyacrylamide gels containing 0.1% gelatin). After electrophoresis, each gel was incubated with 50 ml of zymogram developing buffer for 48 h (37.5 °C) in shaking bath. Then, the gels were stained with coomassie brilliant blue (1%, with 10% acetic acid, 10% isopropyl alcohol, diluted with dd H2O).

2.7. Western blotting

The TGs ipsilaterally to the surgery site and spinal cord tissues were rapidly dissected and homogenized in RIPA Lysis Buffer after the animals deep anesthesia with sodium pentobarbital (4%, i.p. 50 mg/kg). The protein concentrations were determined by BCA Protein Assay (Thermo Fisher, Waltham, MA), and 40 to 80 μg of proteins was loaded and separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes (Millipore Corp., Bedford, MA). The membranes were blocked with 5% bovine serum albumin for 1 h at room temperature, probed with antibodies overnight at 4 °C with the primary antibodies, and then incubated with Horseradish Peroxidase (HRP)-coupled secondary antibodies. The primary antibodies used included β-actin (1:5000), IL-1β (1:1000), TNF-α (1:1000), p-NR1 (1:1000), p-PKCγ (1:1000), p-p65 (Ser536) (1:1000), SOCS3(1:1000). The filters were then developed by enhanced chemiluminescence reagents (PerkinElmer) with secondary antibodies (anti-rabbit or anti-mouse, 1:5000). Data were analyzed with the Molecular Imager (Gel DocTM XR, 170-8170) and the associated software Quantity One-4.6.5 (Bio-Rad Laboratories, Berkeley, CA).

2.8. Statistical analyses

GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA)was used to conduct all the statistical analyses. Alteration of expression of the proteins detected were tested with one-way ANOVA. The behavioral responses were tested with one-way ANOVA and the differences in latency over time among groups were tested with two-way ANOVA. Bonferroni post hoc tests were conducted for all ANOVA models. Results were represented as mean ± SEM of the independent experiments. Results described as significant were based on a criterion

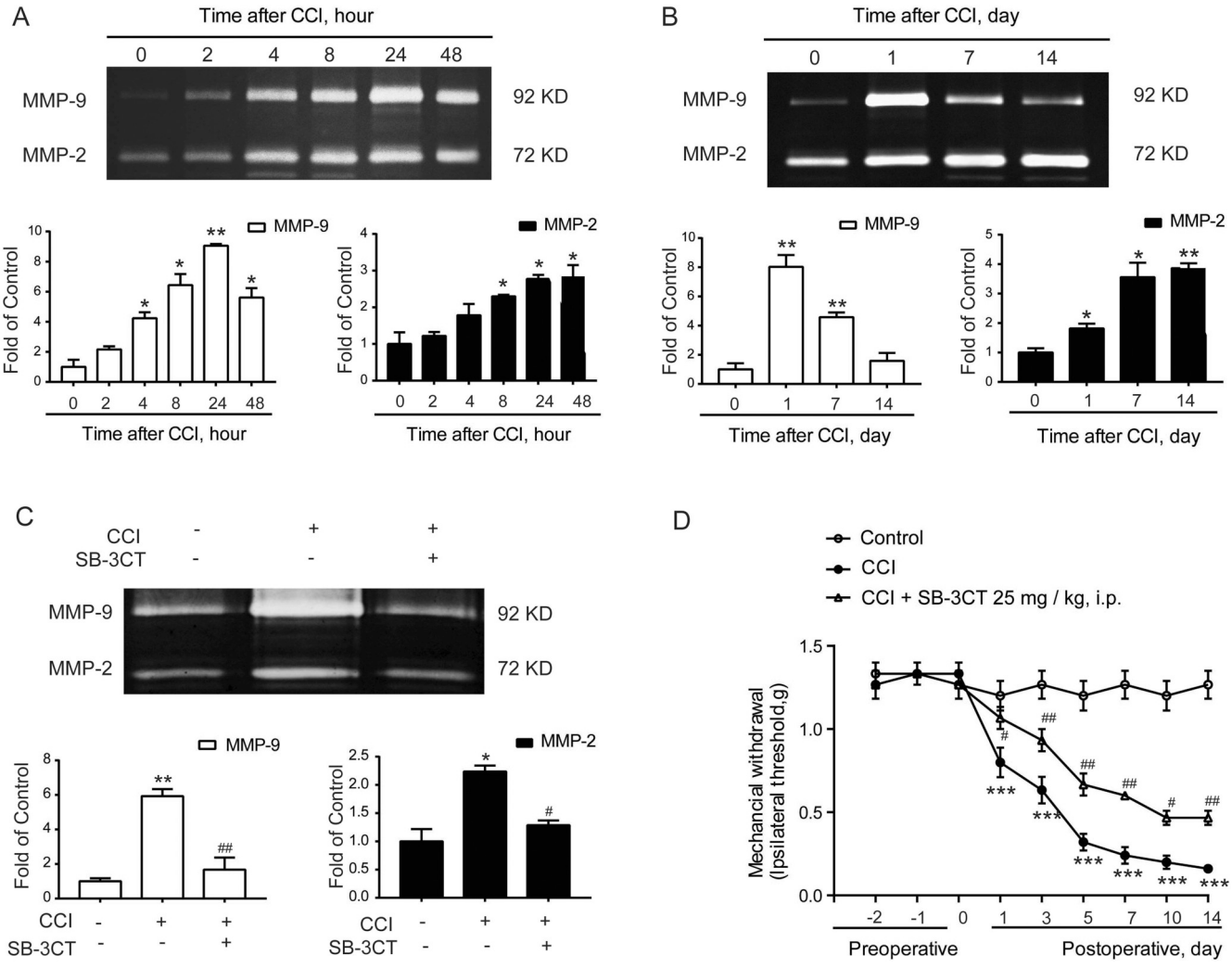

Fig. 1. MMP-9 and MMP-2 showed different temporal upregulation in the TG after CCI and were required for producing neuropathic pain symptom. (A) Gelatin zymography exhibited a time course of MMP-9 and MMP-2 activity in TG of CCI mice within 48 h. (n = 4). (B) Gelatin zymography exhibited a time course of MMP-9 and MMP-2 activity in TG of CCI mice within14 days. (n = 4). (C) Gelatin zymography exhibited the MMP-9/2 selective inhibitor (SB-3CT, 25 mg/kg, i.p.) could effectively suppress CCI-induced MMP-9/2 over-expression. Mice were injected 2 h after CCI surgery, followed by an additional dose at 4 h. The samples were collected at day 1 after CCI surgery(n = 4). (D) The MMP-9/2 selective inhibitor (SB-3CT, 25 mg/kg, i.p.) relieved CCI-induced mechanical allodynia and delayed the formation of pain. Mice received repeated-dose treatment from day 0 to 4 (n = 6). (*P < 0.05, **P < 0.01, ***P < 0.001 vs. Control; #P < 0.05, ##P < 0.01 vs. CCI group).

of $p < 0.05$.

3. Results

3.1. MMP-9 and MMP-2 showed different temporal upregulation in the TG after CCI and were required for the progress of neuropathic pain

To verify the role of MMP -9 and MMP-2 in peripheral sensitization, we firstly examined gelatinase activity of MMP-9 and MMP-2 in TG at different phases after CCI. MMP-9 showed very low activity in the naïve TG and began to increase remarkably 4 h after surgery. However, this upregulation was transient. MMP-9 peaked at day 1 and declined gradually, which almost returned to baseline level at day 14. Conversely, the expression of MMP-2 showed moderate increase after nerve injury, this increase was gradual and continued until the pain formation (Fig. 1A, B). Furthermore, SB-3CT, a novel MMP-9/2 selective inhibitor

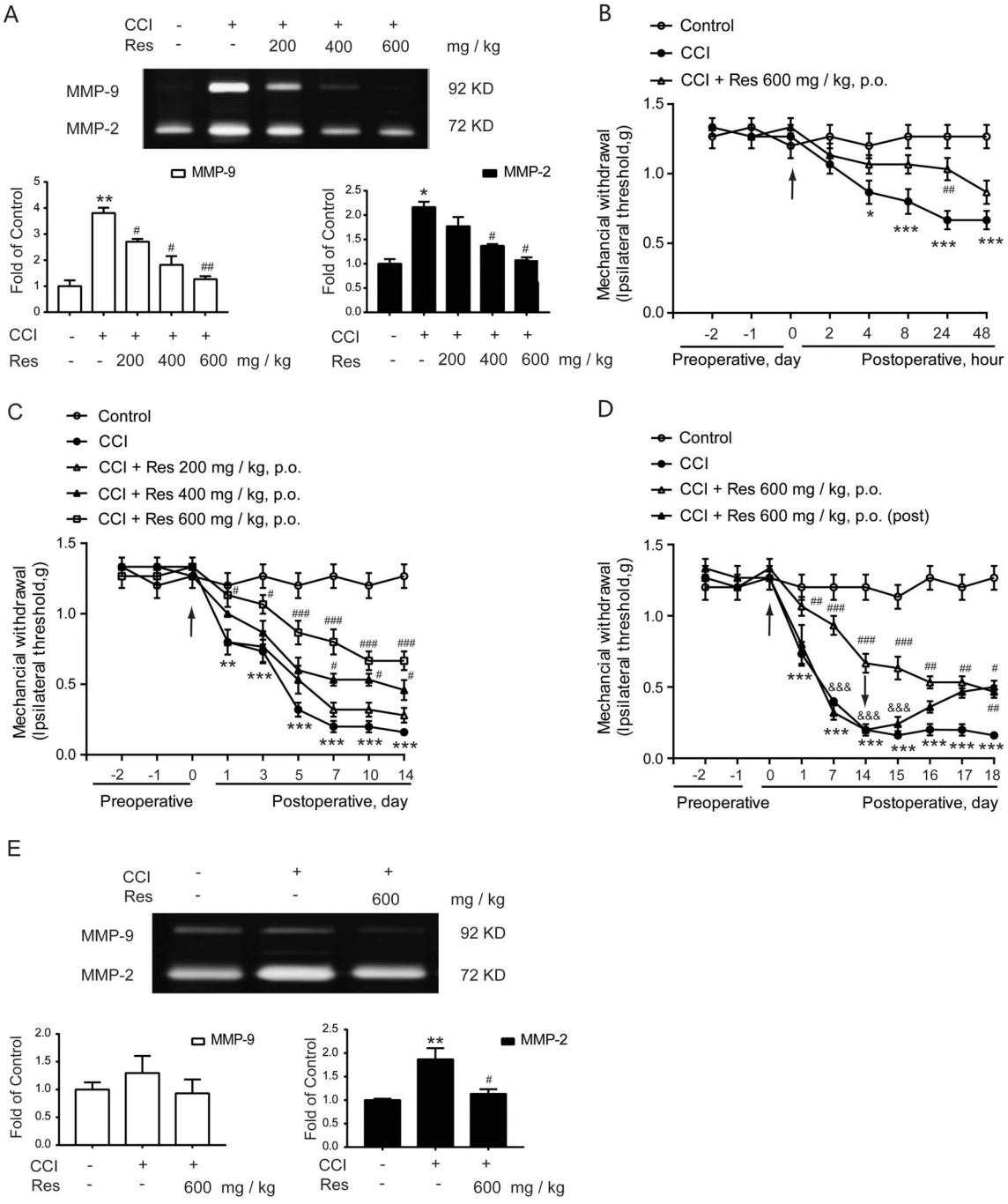

Fig. 2. Pretreatment with resveratrol suppressed CCI-induced activation of MMP-9/2 in TG and attenuated mechanical allodynia. (A) Effects of various doses of resveratrol (200, 400, 600 mg/kg, p.o.) on MMP-9 and MMP-2 activity in TG at 1 day after CCI. (B) Single preadministration of resveratrol (600 mg/kg, p.o.) attenuated CCI-induced mechanical allodynia within 24 h after injury (n = 6). (C) Consecutive administration of resveratrol (200, 400, 600 mg/kg, p.o.) attenuated CCI-induced mechanical allodynia within 14 days after injury (n = 6). (D) Consecutive administration of resveratrol (600 mg/kg, p.o.) before or after pain formation both showed a amelioration of mechanical allodynia after CCI surgery (n = 6). Initial drug administration time is indicated by the arrows. (E) Consecutive administration of resveratrol (600 mg/kg, p.o.) after pain formation could effectively suppress CCI-induced MMP-2 over-expression. The samples were collected at day 18 after CCI surgery(n = 4). (*P < 0.05, **P < 0.01, ***P < 0.001 vs. Control; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. CCI group; &&&P < 0.001 vs. resveratrol-treated group).

effectively suppressed CCI-induced MMP-9/2 over-expression and relieved the mechanical allodynia obviously (Fig. 1C and D). MMP-9/2 levels were measured after SB-3CT administration on day 1 after CCI surgery.

3.2. Pretreatment with resveratrol suppressed CCI-induced activation of MMP-9/2 in TG and attenuated mechanical allodynia

To investigate the effects of resveratrol on MMP-9 and MMP-2 in TG, single preadministration of resveratrol (200, 400, 600 mg/kg, p.o.) was given to CCI mice 30 min before surgery. Gelatin zymography results showed that the activation of MMP-9 and MMP-2 in the mice TG were inhibited by resveratrol in a dose-dependent manner (Fig. 2A). We found that pain-related behaviors appeared obviously 4 h after CCI surgery and single preadministration of resveratrol (600 mg/kg, p.o.) could significantly attenuate this allodynia within 24 h (Fig. 2B). Besides, a marked decrease in withdrawal threshold was observed 5 and 7 days after nerve injury and kept going down until the pain formation in day 14 (Fig. 2C). Notably, the occurrence of CCI-induced neuropathic pain was delayed and those pain-related behaviors were obviously ameliorated by resveratrol in a dose-dependent manner (Fig. 2C). To increase effects of the drug, resveratrol was orally consecutive administrated with 200, 400, and 600 mg/kg, once a day for 5 days right after the CCI procedure. The dose was designed on our prior experience [22]. Besides, pretreatment with resveratrol (600 mg/kg, p.o.) could maintain mild but clear analgesic effects until 18 days after CCI surgery. Interventions at postoperative days ameliorated mechanical allodynia as well (Fig. 2D). Meanwhile, we found that comparing with the low activity MMP-9 exhibited 18 days after CCI, MMP-2 showed relatively more obvious activity in this period and the up-regulation could be reversed by resveratrol (Fig. 2E).

3.3. Pretreatment with resveratrol reduced CCI-induced upregulation of pro-inflammatory factors and phosphorylation of NR1 and PKCγ

Mounting evidence suggests that IL-1β and Tumor necrosis factor (TNF)-α have critical role in the neuroinflammation [6,28–30]. Moreover, IL-1β cleavage and TNF-α activation from its transmembrane precursor are all MMP-dependent processes [8,31]. In this study, we found the rapidly upregulation of IL-1β and TNF-α in TG induced by CCI were reversed by resveratrol (600 mg/kg, p.o.) (Fig. 3A). Given that IL-1β and TNF-α could also serve as neuromodulators in central nervous system (CNS) and induce/enhance synaptic plasticity after peripheral injury [32], we examined their activity in STN and found the expression of IL-1β and TNF-α were remarkably increased after CCI (Fig. 3B). Furthermore, as the vital characteristic of central sensitization, neuronal activity was also examined by western blotting. As shown in Fig.3C, the phosphorylation of the NMDA receptor 1 (NR1) and protein kinase C (PKC)γ were increased in STN after CCI. However, up-regulation of these activated inflammatory participants as well as indicators of the excitability of neurons mentioned above were inhibited after resveratrol pretreatment (600 mg/kg, p.o.) (Fig. 3B,C).

3.4. Pretreatment with resveratrol inhibited MMP-9 and MMP-2 activation via TLR-4/NF-κB signaling pathway in TG by upregulating SOCS3

To further investigate how resveratrol suppressed the MMP-9 and MMP-2 activity in TG, TLR-4/NF-κB signaling pathway, which contributes to the over-expression of MMPs in many cases [13–15], is taken into consideration. We used a TLR-4 antagonist (Cli-095, 1 μg/μl, i.t.) and observed that pretreatment with this inhibitor suppressed CCI-induced MMP-9/2 over-expression (Fig. 4A). Moreover, mechanical allodynia after CCI was ameliorated via TLR-4 signaling pathway blocking (Fig. 4B) and the upregulation of p-p65 were inhibited as well (Fig. 4C). Collectively, regulating TLR-4/NF-κB activity in TG was close

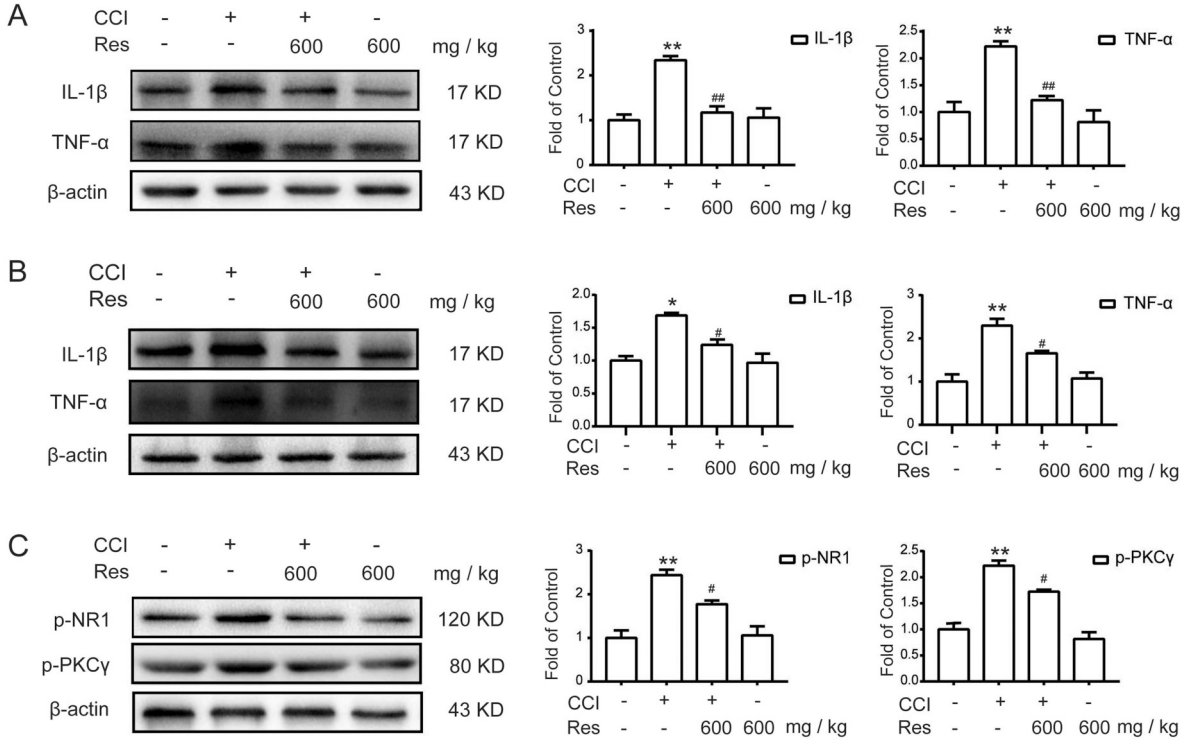

Fig. 3. Pretreatment with resveratrol reduced CCI-induced upregulation of pro-inflammatory factors and phosphorylation of NR1 and PKCγ. (A) Single preadministration of resveratrol (600 mg/kg, p.o.) reversed the upregulation of IL-1β and TNF-α induced by CCI surgery in TG (n = 4). The samples were collected 24 h after CCI surgery. (B) Consecutive administration of resveratrol (600 mg/kg, p.o.) reversed the upregulation of IL-1β and TNF-α induced by CCI surgery in STN (n = 4). (C) Consecutive administration of resveratrol (600 mg/kg, p.o.) inhibited the phosphorylation of PKCγ and NR1 induced by CCI surgery in STN (n = 4). The samples were collected at day 14 after CCI surgery. (*P < 0.05, **P < 0.01 vs. Control; #P < 0.05, ##P < 0.01 vs. CCI group).

related to the MMP-9/2 activation and pain control. Then we tried to explore the relationship between TLR-4/NF-κB signaling pathway and resveratrol. We found that resveratrol was equally capable of inhibiting the upregulation of p-p65 in TG (Fig. 4D) and of interest, resveratrol could upregulate the expression of SOCS3 in a dose-dependent manner (200, 400, 600 mg/kg, p.o.)(Fig. 4E), whereas this effect was reversed by the AMPK inhibitor compound C (30 μg/20 μl, i.t.) significantly (Fig. 4F).

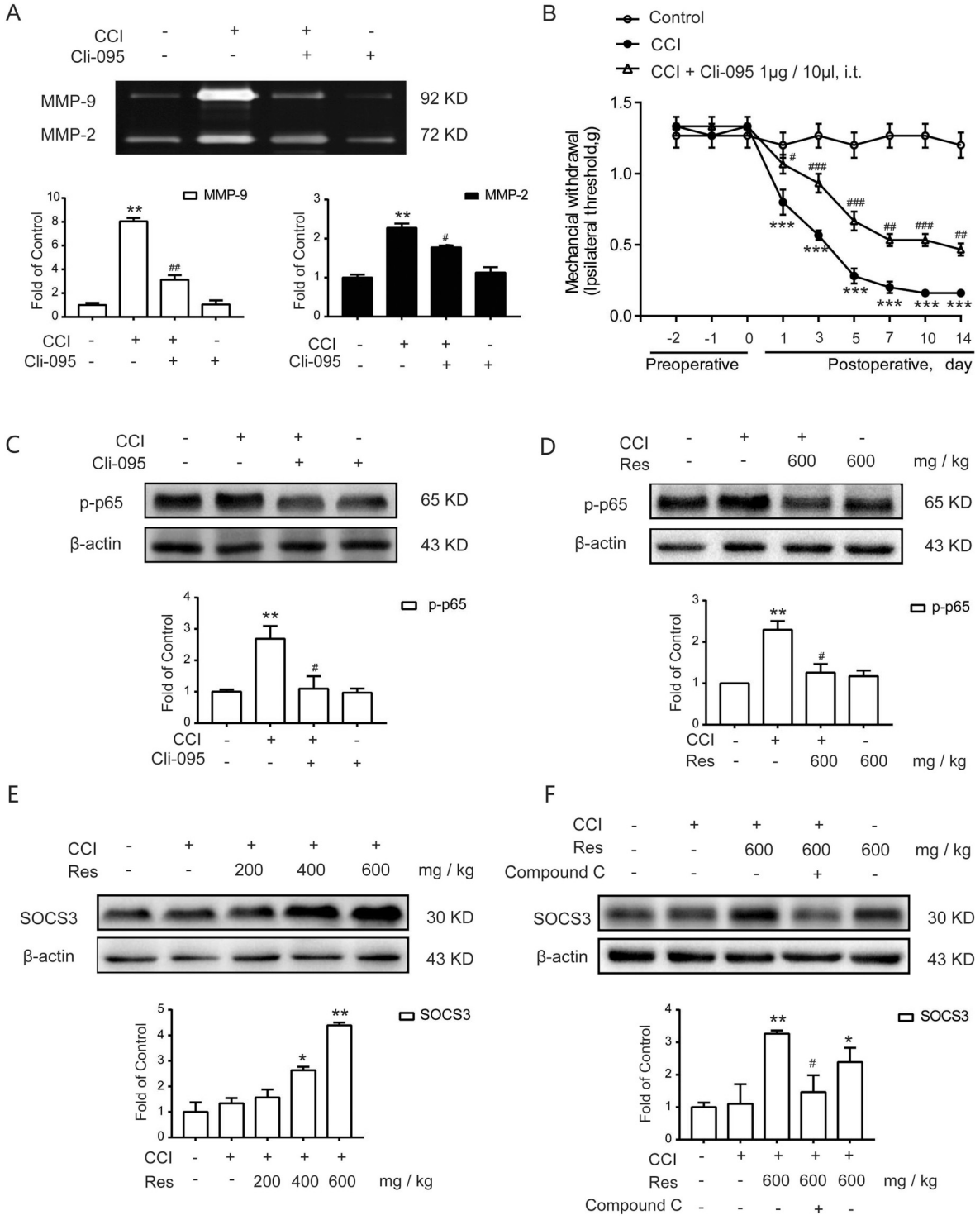

Fig. 4. Pretreatment with resveratrol inhibited MMP-9 and MMP-2 activation via TLR-4/NF-κB signaling pathway in TG by upregulating SOCS3. (A) CCI-induced MMP-9/2 upregulation in TG were reversed by TLR-4 antagonist (Cli-095, 1 μg/μl, i.t.) (n = 4). (B) CCI-induced mechanical allodynia was relieved and delayed by TLR-4 antagonist (Cli-095, 1 μg/μl, i.t.) (n = 6). (C) The upregulation of p-p65 induced by CCI in TG was abrogated by TLR-4 antagonist (Cli-095, 1 μg/μl, i.t.) (n = 4). (D) Pretreatment with resveratrol (600 mg/kg, p.o.) abrogated the upregulation of p-p65 induced by CCI in TG (n = 4). (E) Pretreatment with resveratrol (200, 400, 600 mg/kg, p.o.) induced the expression of SOCS3 in a dose-dependent manner (n = 4). (F) Resveratrol (600 mg/kg, p.o.) upregulated the expression of SOCS3, this effect was reversed by compound C(n = 4). Single administration of resveratrol was given to CCI mice 30 min before surgery. Compound C (30 μg/20 μl, i.t.) was co-administered. All samples were collected and analyzed 24 h after CCI surgery. (*P < 0.05, **P < 0.01, ***P < 0.001 vs. Control; #P < 0.05, ##P < 0.01, ###P < 0.001 vs. CCI group).

4. Discussion

In this study, our findings have revealed that pretreatment with resveratrol could ameliorate CCI-induced neuroinflammation and mechanical allodynia by suppressing the activation of MMP-9/2 in TG, which showed different temporal upregulation after infraorbital nerve ligation and were required for producing neuropathic pain symptom. Moreover, resveratrol exerting its powerful inhibition on the MMP-9/2 activation after CCI may be closely related to TLR-4/NF-κB signaling pathway and the induction of SOCS3.

Neuropathic pain is a clinical challenge since the underlying mechanisms remain poorly understood. In the last several decades, evidences have demonstrated that no matter neuronal plasticity but also neuroinflammation play a vital role in the pathological process [6,33,34]. The connections between neuropathic pain and inflammation are intimately. It is generally believed that a heightened perception of pain after nerve injury is related to central sensitization [35–37], which refers to enhanced responses of pain circuits and happens in response to the further aggravation of neuroinflammation in peripheral nervous system [6]. Continued peripheral inflammation leads to the hyperactivity of primary sensory neurons, which increase the release of neurotransmitters and neuromodulators from the central terminals of primary afferents and evoking excessive neuronal activity in the spinal cord and trigeminal nucleus, i.e. central sensitization. Considering that microvascular compression and demyelination are primary etiologies in most cases of TN [38], controlling peripheral inflammation in TG is expected to be an effective therapeutic strategy.

MMP-9 and MMP-2 are widely studied proinflammatory participants and could be activated by each other as well as by free radicals produced by hypoxia. An ischaemic episode such as CCI could trigger the upregulation of MMP-2 and MMP-9 by endothelial cells, pericytes and other components of the neurovascular epithelium [39,40]. Accumulating evidence indicates that they are involved in the induction and development of neuroinflammation [36,41,42]. Inhibiting MMP-9/2 activity could attenuate neuroinflammation and mechanical allodynia in the spinal nerve ligation (SNL) model of neuropathic pain [43]. Consistently, in our study, inhibition of MMP-9/2 activity by the selective inhibitor corroborated well with attenuation of CCI-induced pain sensitization obviously (Fig. 1C and D), which further demonstrate their potent roles in neuropathic pain. Of note, a time course of MMP-9 and MMP-2 activity in the TN mice model was rarely mentioned previously. In our study, MMP-9 increased remarkably 4 h after surgery, peaked at day 1 and almost returned to baseline level at day 14. Meanwhile, the expression of MMP-2 showed moderate increase right after nerve injury, which was gradual and continued until the pain formation (Fig. 1A, B).

Evidence shows the highly inducible MMP-9 in early stages of inflammation could damage basement membrane, upregulate cytokines and exacerbate epineurial oedema [44,45]. It is possible that the dramatic increase of MMP-9 activity right after injury appears to correlate with the pain-related behaviors occurring initially. In our research, mechanical allodynia began to appear obviously 4 h after CCI surgery (Fig. 2B), corresponding to MMP-9 upregulation (Fig. 1A). Meanwhile, a marked decrease in withdrawal threshold observed 7 days after CCI surgery (Fig. 2C, D) may be tightly associated with the over-expressed MMP-2 later on (Fig. 1B), which greatly facilitate macrophage infiltration during these days [41]. Therefore, we tend to believe that in TN models, MMP-9 and MMP-2 are both essential for the development of TN and MMP-9 is more indispensable in the early stages of neuroinflammation. Whereas, the maintenance of neuropathic pain depends on the delayed but persistent upregulation of MMP-2.

Based on these findings, interventions in early stages were taken and not surprisingly, we found that pretreatment with resveratrol, a natural anti-inflammatory compound, significantly suppressed the activity of MMP-9 and MMP-2 in a dose-dependent manner (Fig. 2A). Moreover, resveratrol was able to attenuate and delay CCI-induced mechanical allodynia remarkably (Fig. 2B-D). Therefore, we realized resveratrol could act as a dual inhibitor to both MMP-9/2, which help to relieve neuropathic pain during whole phases. Although treatment with resveratrol at postoperative days ameliorated mechanical allodynia as well (Fig. 2D), we should bear in mind that preadministation of drugs greatly improved the quality of life of sufferers during the entire period after injury.

IL-1β and TNF-α are critical participants in the neuroinflammation, which could then activate a positive feedback loop and lead to inflammatory cascades [6,28–30,46]. Moreover, IL-1β could be a critical substrate of MMP-9 and the availability of both TNF-a and its TNFRI receptor was controlled by MMPs [8,31]. Regulating MMPs activity controlled the expression of IL-1β and then affected the development of inflammation [47]. In our study, IL-1β and TNF-α were highly inducible in TG right after CCI surgery and this over-expression was inhibited after resveratrol pretreatment (Fig. 3A). These findings supported our consideration that in the early stages, resveratrol greatly alleviated peripheral neuroinflammation by mainly inhibiting the activity of MMP-9/2. Besides, the suppression of MMP-9/2 activation and the alleviation of peripheral neuroinflammation later on helped to further ameliorate central sensitization (Fig. 3B and C). Evidence shows that after nerve injury, *N*-methyl-D-aspartate (NMDA) receptor-dependent synaptic plasticity contributes to enhanced sensory responses. The activation of NMDA receptors like NR1 induces $Ca2+$ influx and then activates calcium-sensors such as CaMKII [48]. These activatory proteins can phosphorylate downstream molecules (eg, PKCγ), which in turn leads to further activation of NMDA receptors and initiates prolonged enhancement of the excitability of neurons [49]. In this study, NR1and PKCγ were used as indicators of the excitability of neurons. Since resveratrol inhibited the expression of inflammatory factors such as IL-1β, TNF-α, MMPs and reduced peripheral inflammation after CCI in our study, and evidence showed suppressing IL-1β and MMPs could down-regulate NMDAR-mediated intracellular calcium increase and synaptic plasticity [50,51], we speculate that resveratrol subsequently down-regulated central sensitization, including the phosphorylation of NR1 and PKC-γ.

A growing body of evidence suggests that TLR-4/NF-κB signaling pathway is intimately associated with the regulation of MMP-9/2 and neuroinflammation [13–15]. High glucose-induced inflammatory response was suppressed via inhibition of TLR-4/NF-κB/MMP-9 axis [52]. In neuropathological conditions, TLR-4 which could be expressed in variety of cells like macrophages and neuroglia cells is able to detect components of foreign pathogens (PAMPs, pathogen-associated molecular patterns) and endogenous ligands (DAMPs, danger-associated molecular patterns) released by stressed cells, then trigger downstream signaling pathways fast and generate plenty of proinflammatory mediators [53,54]. Other researches show HMGB1 (high-mobility group box 1 protein), which belongs to DAMPs, is a ubiquitous nuclear protein released by glia and neurons upon inflammasome activation and acts as an endogenous ligand of TLR-4. It is noteworthy that mechanical injury could lead to the release of HMGB1 from stressed cells [55,56]. Thus, we speculate that in CCI model, when the nerve injury happens, HMGB1/TLR-4/NF-κB axis was a potential major pathway of MMP-9/2 expression and a key initiator of neuroinflammation. Our study showed that CCI-induced MMP-9/2 upregulation, p-p65 nuclear translocation and mechanical allodynia were inhibited by blocking TLR-4 signaling pathway (Fig. 4A, B and C). Furthermore, pretreatment with resveratrol effectively inhibited the phosphorylation of p65 (Fig. 4D) and suppressed MMP-9/2 activation (Fig. 2A). Combined with these findings mentioned above, we believe that MMP-9/2 participating in pain sensitization could be regulated by resveratrol via TLR-4/NF- κ B signaling pathway after peripheral nerve injury.

It's a pity that there is no safe and effective TLR-4 inhibitor available in clinic for the treatment nowadays. Notably, Yoshimura et al. reported TLR-4/NF-κB signaling pathway could be negatively regulated by SOCS3 [21]. SOCS3 is an endogenous anti-inflammatory protein and

able to inhibit NF-κB-dependent transcription by inhibiting the association between TNF receptor-associated factor 6 (TRAF6) and TGF-β-activated kinase 1 (TAK1), both of which are served as key molecules involved in TLR-4/NF-κB signal cascade [21]. SOCS3 could interact with p65 and induce its ubiquitin-dependent proteasomal degradation as well [57]. Therefore, SOCS3 could be intimately related with TLR-4/NF-κB signaling pathway.

Evidence shows resveratrol was able to up-regulate SOCS3 production in LPS activated microglia and exerted neuroprotective effects [58]. Consistently, in our study, it is intriguing that resveratrol could upregulate the expression of SOCS3 in a dose-dependent way as well (Fig. 4E), whereas this effect was reversed by the AMPK (AMP-activated protein kinase) inhibitor compound C significantly (Fig. 4F). AMPK is a ubiquitous kinase endogenously activated by AMP and ADP, it is regarded as a regulator of neuronal function and plays a vital role in the management of pain, especially neuropathic pain [59,60]. Evidence shows neuroinflammation associated with morphine tolerance could be suppressed via AMPK-SOCS3 dependent way in the spinal cord [61]. As a widely recognized stimulant of AMPK, resveratrol could suppress glial activation and alleviate trigeminal neuralgia via activation of AMPK in our previous study [22]. In this study, the increased expression of SOCS3 was reversed by the AMPK inhibitor compound C (Fig. 4F). Hence, resveratrol increased SOCS3 expression via AMPK-mediated signaling pathway.

Taken together, combined with our study we speculate that SOCS3 may participate in the process of which resveratrol negatively regulates TLR-4/NF-κB signaling pathway and inhibits the expression of MMP-9/2. Furthermore, what was in agreement with our speculation is that SOCS1, another member of SOCS family which exerted negative regulation on both cytokine receptor and TLR-mediated signaling, also took part in the neuroprotective effects of resveratrol by suppressing inflammatory response. Resveratrol upregulated SOCS1 expression which blocked the activation of NF-κB as well as MAPK families and interfered with signal transduction, finally counteracted microglial inflammation.

However, it is noteworthy that even though this view based on plenty of facts is novel and reasonable, revealing more details about the interlacing molecular pathway in which resveratrol suppresses CCI-induced MMP-9/2, more rigorous and detailed experimental design needs to be completed.

In summary, the significance of this research lied in that the upregulation of MMP-9/2 were firstly examined in TG after CCI surgery. Moreover, the capacity of resveratrol on alleviating neuroinflammation and pain sensitization was attributed to the negative regulation on TLR-4/NF-κB/MMP-9/2 signaling pathway, which may closely relate to the upregulation of SOCS3. This new perspective of targeting early pathological stages of neuroinflammation may represent a promising avenue for the effective prevention and treatment of TN.

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Not applicable

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Yuling Yin designed and performed experiments, analyzed data, and wrote the manuscript. Rong Guo, Yu Shao, Mixue Ge, Chen Miao and Ling Cao performed experiments and analyzed data. Yanjing Yang and Liang Hu supported and supervised the overall project and final approval of the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

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