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The nitroxyl donor, Angeli's salt, reduces chronic constriction injury-induced neuropathic pain

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ABSTRACT

Chronic pain is a major health problem worldwide. We have recently demonstrated the analgesic effect of the nitroxyl donor, Angeli's salt (AS) in models of inflammatory pain. In the present study, the acute and chronic analgesic effects of AS was investigated in chronic constriction injury of the sciatic nerve (CCI)-induced neuropathic pain in mice. Acute (7th day after CCI) AS treatment (1 and 3 mg/kg; s.c.) reduced CCI-induced mechanical, but not thermal hyperalgesia. The acute analgesic effect of AS was prevented by treatment with 1H-[1,2, 4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ, a soluble guanylate cyclase inhibitor), KT5823 (an inhibitor of protein kinase G [PKG]) or glibenclamide (GLB, an ATP-sensitive potassium channel blocker). Chronic (7-14 days after CCI) treatment with AS (3 mg/kg, s.c.) promoted a sustained reduction of CCI-induced mechanical and thermal hyperalgesia. Acute AS treatment reduced CCI-induced spinal cord allograft inflammatory factor 1 (known as Iba-1), interleukin-1 β (IL-1 β), and ST2 receptor mRNA expression. Chronic AS treatment reduced CCI-induced spinal cord glial fibrillary acidic protein (GFAP), Iba-1, IL-1 β , tumor necrosis factor- α (TNF- α), interleukin-33 (IL-33) and ST2 mRNA expression. Chronic treatment with AS (3 mg/kg, s.c.) did not alter aspartate aminotransferase, alanine aminotransferase, urea or creatinine plasma levels. Together, these results suggest that the acute analgesic effect of AS depends on activating the cGMP/PKG/ATP-sensitive potassium channel signaling pathway. Moreover, chronic AS diminishes CCI-induced mechanical and thermal hyperalgesia by reducing the activation of spinal cord microglia and astrocytes, decreasing TNF- α , IL-1 β and IL-33 cytokines expression. This spinal cord immune modulation was more prominent in the chronic treatment with AS. Thus, nitroxyl limits CCI-induced neuropathic pain by reducing spinal cord glial cells activation.

Keywords: Nitroxyl donor; Neuropathic pain; Astrocytes; Microglia; Cytokines.

1. Introduction

Pain is a complex response by the organism to maintain body integrity. Chronic pain conditions such as neuropathic pain arise as a consequence of a lesion or disease of the somatosensory system, leading to the perpetuation of pain symptoms [1–3]. Spontaneous behavior in response to normally innocuous tactile stimuli (allodynia) and exaggerated pain in response to noxious stimuli (hyperalgesia) characterize neuropathic pain [2,4,5]. The pathogenesis of neuropathic pain is characterized by reorganization of the spinal cord due to changes in synaptic transmission and activation of neurons and glial cells such as astrocytes and microglia [6–8]. Once activated, these cells release several pro-inflammatory/nociceptive cytokines [9] that activate and/or sensitize nociceptive neurons in the spinal cord, further contributing to pathological pain [4]. Furthermore, cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-33 (IL-33) induce the sensitization and hyperexcitability of nociceptive neurons [10–14]. Notably, chronic pain represents a therapeutic challenge for clinicians due to severity, frequency, limited effective treatment and several side effects [1,2,15,16].

Nitroxyl or nitrosyl hydride (HNO) is one-electron more reduced than nitric oxide (NO). Importantly, nitroxyl exhibits different chemical and pharmacological properties compared to NO [17–19] and reactive oxygen and other nitrogen species [20]. Although nitroxyl and NO are different chemical entities, they nonetheless, can activate similar signaling pathways. For instance, nitroxyl can activate soluble guanylate cyclase (sGC), a primary NO target [21–24]. As such, the nitroxyl donor, Angeli's salt ($\text{Na}_2\text{N}_2\text{O}_3$; AS), reduces inflammatory hyperalgesia and spontaneous overt pain-like behavior by activating the cGMP/PKG/ATP-sensitive potassium channels [25,26]. The analgesic effect of AS depends on nitroxyl since it was inhibited by L-cysteine (a nitroxyl scavenger) [25,26].

AS significantly reduces the acute inflammatory hyperalgesia induced by carrageenan, lipopolysaccharide (LPS), TNF- α , IL-1 β , and prostaglandin E₂ as well as diminishes the overt pain-like behavior induced by acetic acid, phenyl-p-benzoquinone and formalin [25,26]. However, it is not known whether AS exerts analgesic activity capable of controlling neuropathic pain. Thus, the acute and chronic analgesic effects of AS on chronic constriction injury of the sciatic nerve (CCI) in mice were evaluated. Furthermore, whether the analgesic effect of AS depends on activating the cGMP/PKG/ATP-sensitive potassium channel pathway and modulating spinal cord glial cell activity (astrocytes and microglia), and cytokines (TNF- α , IL-1 β , and IL-33/ST2) was tested.

2. Material and methods

2.1. Animals

All experiments were performed with the approval of the protocol by the Ethics Committee for Animal Research of the Universidade Estadual de Londrina (UEL) under the protocol CEUA-UEL 26292.2012.91, and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [27] and International Association for the Study of Pain (IASP). Experiments were conducted using male Swiss mice (20-25 g) obtained from the Universidade Estadual de Londrina's animal facility that were housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 06:00 a.m.) with access to food and water *ad libitum*. The mice were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments.

2.2. Chronic constriction injury (CCI) of the sciatic nerve

Mice were anaesthetized and maintained with volatile anesthetic isoflurane (3% in O₂). Surgical procedures were performed as previously described [28] with modifications [14]. Briefly, the distal portion of the sciatic nerve was tied with surgical thread (catgut 4-0). In sham-operated mice, the sciatic nerve was exposed without ligation. The wound was closed and covered with iodine solution.

2.3. Electronic pressure meter test of mechanical hyperalgesia

Mechanical hyperalgesia was tested in mice as previously reported [29]. Briefly, the test consists of evoking a hind paw flexion reflex with a hand-held force transducer (electronic von Frey anesthesiometer; Insight, Ribeirão Preto, SP, Brazil) adapted with a 0.5 mm² contact area polypropylene tip. The investigator was trained to apply the tip perpendicularly to the central area of the hind paw, and the end point was characterized by the removal of the paw. The intensity of the hyperalgesia was quantified as the change in pressure applied by subtracting the mean of the 3 values obtained after the surgery from the mean of the three values observed in the day before surgery.

2.4. Hot plate test

Mice were placed in the hot plate apparatus (EFF 361; Insight, Ribeirao Preto, SP, Brazil) maintained at 52 °C [30]. The first ipsilateral hind paw flexion reflex (nociceptive behavior) was registered. The response latency was recorded in the day before the surgery and on 7 and 7-14 days post-surgery for the experiments evaluating thermal hyperalgesia and effect of AS in acute and chronic protocols, respectively. The maximum latency (cut-off) was set at 20 s to avoid tissue damage [31].

2.5. RT-PCR and quantitative PCR

Spinal tissue samples were homogenized in TRIzol® reagent (Life Technologies), and total mRNA were isolated according to manufacturer's directions. RNA purity was confirmed by the 260/280 ratio [32]. RT-PCR and quantitative PCR were performed using GoTaq® 2-Step RT-qPCR System (Promega) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems®). The primer sequences shown in Table 1 were used, and their values were calculated as fold change relative to control after normalization to the GAPDH (glyceraldehyde 3-phosphate dehydrogenase) gene [33–37].

2.6. Hepatotoxicity and nephrotoxicity

Plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), urea and creatinine were used as indicators of hepatotoxicity [38] and nephrotoxicity [39], respectively. These assays were performed using a diagnostic kit from Labtest (Lagoa Santa, Minas Gerais, Brazil) in samples collected in the 14th day after surgery after behavioral evaluation.

2.7. Drugs

Drugs were obtained from the following sources: Glibenclamide (GLB) and KT5823 were obtained from Sigma Aldrich (St Louis, MO, USA). 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) was purchased from Calbiochem (San Diego, CA, USA). AS was synthesized and used as previously described [40]. The stability of stock solutions prepared in 10 mM NaOH and stored at 20°C was determined from the extinction coefficient at 250 nm (ϵ of 8000 M⁻¹ cm⁻¹ for AS) [41]. AS was prepared at 7 mg/ml of 10 mM NaOH; ODQ were diluted in

dimethylsulfoxide (DMSO) 2% in saline, KT5823 in DMSO 2% or GLB in Tween 80 5%. AS, ODQ, KT5823 or GLB solutions were re-diluted in sterile saline for *in vivo* administration.

2.8. Protocols

Mice were treated with AS (s.c.; dose range: 0.3-3 mg/kg; diluted in 10 mM NaOH plus saline) at the 7th day after CCI surgery, followed by evaluation of mechanical and thermal hyperalgesia (1, 3, 5, 7 and 24 h after AS treatment). Next, pharmacological treatments targeting guanylate cyclase (ODQ, a soluble guanylate cyclase inhibitor, 0.3 mg/kg, i.p., 30 min before AS treatment, diluted in 50 μ l of DMSO 2% in saline), protein kinase G (PKG) (KT5823, an inhibitor of PKG, 0.5 μ g/mouse, i.p., 5 min before, diluted in 50 μ l of DMSO 2% in saline) and ATP-sensitive potassium channel (GLB, an ATP-sensitive potassium channels blocker, 0.3 mg/kg, i.p., 45 min before, diluted in 50 μ l of Tween 80 5% in saline) were performed at the 7th day after CCI, and mechanical hyperalgesia was evaluated 1, 3, 5, 7 h after AS acute treatment (3 mg/kg, s.c.). In another experiment, mice were chronically treated with AS (3 mg/kg, s.c.) between 7-14 days after CCI surgery, and mechanical and thermal hyperalgesia were evaluated daily. At 5 h after acute (at the 7th day after CCI surgery) or chronic (between 7-14 days after CCI surgery) treatment, spinal cord samples were collected to analyze the pro-IL-1 β , TNF- α , IL-33, ST2, glial fibrillary acidic protein (GFAP), and allograft inflammatory factor 1 (AIF1; also known as Iba-1) mRNA expression by qPCR. Plasma levels of AST, ALT, urea and creatinine were measured to evaluate possible hepatotoxicity and nephrotoxicity of chronic treatment with AS (3 mg/kg, s.c.).

2.9. Statistical analysis

The results are representative of two independent experiments and are presented as the mean $\pm$ SEM ($n = 6$ per group per experiment). Data were analysed using the software GraphPad Prism 6.01. Two-way repeated measure analysis of variance (ANOVA) followed by Tukey's *post hoc* was used to compare all groups and doses at all times when responses were measured at different time points after the stimulus injection. When an analysis was performed at a single time point, one-way ANOVA followed by Tukey's *post hoc* was used. Statistical differences were considered significant when $P < 0.05$.

3. Results

3.1. Single AS treatment reduces CCI-induced mechanical hyperalgesia, but not thermal hyperalgesia.

In the 7th day after CCI surgery mice were treated with AS (s.c. bolus injection; 0.3, 1 and 3 mg/kg). AS reduced CCI-induced mechanical hyperalgesia (Fig. 1A) with significant effect at 5 h with 1 mg/kg of AS and between 1-7 h after the treatment with 3 mg/kg of AS. The antinociceptive effect of AS was maximal at 5 h after treatment with 3 mg/kg of AS, which was chosen for subsequent experiments. AS did not alter CCI-induced thermal hyperalgesia (Fig. 1B) after the acute single treatment.

3.2. Single AS treatment reduces CCI-induced mechanical hyperalgesia by activating the cGMP/PKG/ATP-sensitive potassium channel pathway.

In the 7th day after CCI surgery, mice were treated with ODQ, KT5823 or GLB, followed by acute single treatment with AS (3 mg/kg, s.c.) or vehicle (diluted in 10 mM NaOH plus saline).

Mechanical hyperalgesia evaluation was performed 1, 3, 5, 7 h after AS treatment (Fig. 2A, B, C). The ODQ treatment at 3 and 5 h (Fig. 2A) and KT5823 and GLB treatments at 5 h (Fig. 2B, C) prevented the antinociceptive effect of AS. The inhibitors ODQ, KT5823 and GLB did not alter the mechanical hyperalgesia induced by CCI per se (Fig 2A, B, C).

3.3. AS chronic treatment reduces CCI-induced mechanical and thermal hyperalgesia

Chronic (between 7-14 days after CCI surgery) treatment with AS (3 mg/kg, s.c.) promoted a sustained reduction of CCI-induced mechanical (Fig. 3A) and thermal (Fig. 3B) hyperalgesia.

3.4. AS reduces CCI-induced spinal cord glial cell activation.

CCI induced a significant increase of GFAP (Fig. 4A) and Iba-1 (Fig. 4B) mRNA expression at 7 and 14 days. A single treatment with AS did not alter CCI-induced GFAP mRNA expression, but significantly reduced that of Iba-1. The effect of AS on GFAP and Iba-1 mRNA expression was more pronounced in the chronic treatment protocol with significant reduction of both markers. Therefore, it indicates that AS reduces CCI-induced activation of astrocytes and microglia in the spinal cord, and that its effects were more pronounced upon chronic treatment compared to single acute treatment (Fig. 4A, B).

3.5. AS reduces CCI-induced spinal cord up-regulation of pro-IL-1 β , TNF- α , IL-33 and ST2 receptor mRNA expression.

CCI induced significant increase of pro-IL-1 β , TNF- α , IL-33 and IL-33 receptor (ST2) mRNA expression at 7 (acute single treatment) and 14 (chronic treatment) days after surgery compared to sham group (Fig. 5). Single AS treatment reduced pro-IL-1 β (Fig. 5A) and ST2 (Fig. 5D) mRNA expression without affecting TNF- α (Fig. 5B) and IL-33 (Fig. 5C) mRNA expression at

the 7th day after surgery. Importantly, AS reduced CCI-induced increase in pro-IL-1 β , TNF- α , IL-33 and ST2 mRNA expression in the chronic treatment protocol in the 14th day after surgery (Fig. 5). Therefore, CCI-induced pro-hyperalgesic cytokine mRNA expression in the spinal cord was reduced by AS treatment.

3.6. Chronic AS treatment does not affect aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine plasma levels.

Plasma levels of AST, ALT, urea and creatinine were not altered by chronic treatment (between 7-14 days after CCI surgery) with AS (3 mg/kg, s.c.) (Fig. 6). Therefore, AS seems to be a safe compound regarding liver and renal damage.

4. Discussion

Chronic pain conditions such as neuropathic pain are major health problems worldwide due to their endurance, poor availability of efficacious therapies and undesired treatment side effects. Therefore, developing novel therapeutic strategies is necessary. Peripheral nerve injury such as CCI leads to mechanical and thermal hyperalgesia [42–45] and also provokes a reaction in peripheral immune and spinal cord glial cells [46]. The current study showed that AS reduced CCI-induced neuropathic pain. Acute AS treatment inhibited mechanical hyperalgesia, and chronic treatment with AS reduced both mechanical and thermal hyperalgesia. The acute mechanisms of AS depend on triggering the cGMP/PKG/ATP-sensitive potassium channel signaling pathway, and reducing microglia activation and IL-1 β mRNA expression. The chronic effect of AS treatment involved the reduction of CCI-induced glial cell (astrocytes and

microglia) activation and the mRNA expression for molecules involved in enhancing nociceptive signaling in neuropathic pain such as IL-1 β , TNF- α , IL-33/ ST2 in the spinal cord.

Acute and chronic AS treatments diminished the mechanical hyperalgesia while only chronic treatment was capable of reducing CCI-induced thermal hyperalgesia. Furthermore, inhibitors of the cGMP/PKG/ATP-sensitive potassium channel pathway reversed the acute analgesic effect of AS in the CCI model. Nitroxyl is capable of activating sGC to produce cGMP in other systems [22–24]. In agreement with the AS-induced activation of sGC, AS reduces acute inflammatory pain by activating the cGMP/PKG/ATP-sensitive potassium channels signaling pathway [25,26]. Notwithstanding, there are conflicting literature data showing that the activation of cGMP mediates hyperalgesia in neuropathic pain models [47,48].

Activation of spinal cord microglia dominates the early glial response in the central nervous system (CNS) to peripheral nerve injury as observed by the activation and proliferation of astrocytes [4,49]. Reactive astrogliosis has been shown to correlate with the severity of peripheral nerve injury and increased GFAP expression, which is used as a marker of altered morphology and activity [44,46,49,50]. Microgliosis correlates with increased expression of CD11b and allograft inflammatory factor 1 (AIF1; also known as Iba-1) [46,51]. AS chronic (7–14th days after CCI) treatment diminished both CCI-induced spinal glial cell markers GFAP and Iba-1. On the other hand, the acute treatment with AS (single s.c. injection) only diminished Iba-1 gene expression. Together, a fast initiation of acute AS analgesia and pronounced effect of chronic AS treatment to reduce CCI-induced hyperalgesia and spinal cord neuroinflammation corroborates the therapeutic potential of this nitroxyl donor.

Peripheral nerve injury induces microglia and astrocytes activation in the dorsal horn of the spinal cord. This cellular activation induces and depends on the release of pro-inflammatory

mediators such as cytokines TNF- α , IL-1 β and IL-33 that modulate pain processing [4,6,14,52,53]. In fact, pro-inflammatory cytokines can induce or facilitate neuropathic pain [6,54,55]. Furthermore, mounting evidence showed the augmented expression of TNF- α and IL-1 β at the mRNA and protein levels in the spinal cord after peripheral nerve injury [56–60] or spinal nerve injury [61–64].

IL-33/ST2 signaling also mediates the neuropathic pain induced by CCI and spinal nerve ligation. The spinal cord oligodendrocytes-derived IL-33 activates phosphoinositide-3-kinase (PI3K), mTOR, MAP kinases and nuclear factor kappa B (NF κ B) resulting in the production of TNF- α and IL-1 β , and activation of spinal cord microglia and astrocytes [14]. IL-33 also mediates cytokine production in innate and adaptive inflammatory pain [32,65]. Chronic AS treatment reduced CCI-induced TNF- α , IL-1 β , IL-33, and ST2 mRNA expression as well as GFAP and Iba-1 mRNA expression in the spinal cord. Therefore, it is reasonable that AS acts by inhibiting CCI-induced spinal cord cytokine production and glial cell activation. Of note, AS inhibited only the mechanical hyperalgesia in the acute single treatment, and both mechanical and thermal hyperalgesia in the chronic treatment. A greater inhibition of spinal cord cytokine production and glial cell activation was also observed in the chronic (GFAP, Iba-1, TNF- α , IL-1 β , IL-33, and ST2) treatment compared to the acute (Iba-1, IL-1 β , and ST2) treatment with AS. Thus, it is possible that this greater inhibitory effect in the chronic treatment than in the acute treatment with AS explains the inhibition of mechanical and thermal hyperalgesia in the chronic AS treatment.

5. Conclusions

In conclusion, acute AS treatment inhibits CCI-induced mechanical hyperalgesia by activating the cGMP/PKG/ATP-sensitive potassium channel signaling pathway and inhibiting spinal cord microglia activation and IL-1 β and ST2 mRNA expression. Chronic AS treatment inhibits CCI-induced mechanical and thermal hyperalgesia by inhibiting spinal cord microglia and astrocytes activation; and TNF- α , IL-1 β , IL-33 and ST2 mRNA expression. Altogether, the prominent analgesic effect of AS in neuropathic pain suggests nitroxyl donors as promising therapeutic approaches for the control of chronic neuropathic pain states.

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Conflict of Interest

The authors have no conflict of interest to declare.

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LEGENDS

Table 1: Primer sequences for qPCR

Figure 1. Acute AS treatment reduces mechanical hyperalgesia, but not thermal hyperalgesia induced by CCI in mice. Seven days after CCI, mice were treated with AS by subcutaneous route (s.c.; dose range: 0.3–3 mg/kg) and mechanical (Panel A) and thermal (Panel B) hyperalgesia were evaluated (1, 3, 5, 7 and 24 h after AS treatment, Panel A and Panel B, respectively). n=6 per group per experiment, representative of two experiments. *P<0.05 compared to sham group; **P<0.05 CCI + AS 1 mg/kg compared to CCI + 3 mg/kg AS and #P<0.05 CCI + 3 mg/kg AS compared to CCI + vehicle group. Two-way ANOVA followed by Tukey's *post hoc*. Panel B: BS = thermal threshold before surgery; PS = thermal threshold post-surgery.

Figure 2. AS reduces CCI-induced mechanical hyperalgesia by activating the cGMP/PKG/ATP-sensitive potassium channel pathway. Pharmacological treatments targeting guanylate cyclase (ODQ; 0.3 mg/kg, i.p., 30 min before AS treatment, Panel A), PKG (KT5923; 0.5 µg/mouse, i.p., 5 min before, Panel B) and ATP-sensitive potassium channel (glibenclamide-GLB; 0.3 mg/kg, i.p., 45 min before, panel C) were administrated before AS treatment, and 1, 3, 5, 7 h after AS acute treatment (3 mg/kg, s.c.) mechanical hyperalgesia was evaluated. The inhibitors (ODQ, KT5823 and GLB) did not alter the hyperalgesia per se (Panel A, B and C). n=6 per group per experiment, representative of two experiments. *P<0.05 compared to sham group and #P<0.05 compared to CCI + vehicle group and **P<0.05 compared to CCI + 3 mg/kg AS group. Two-way ANOVA followed by Tukey's *post hoc*.

Figure 3. Chronic AS treatment reduces the mechanical and thermal, hyperalgesia induced by CCI in mice. Seven days after CCI, mice were chronically treated (up to 14 days after surgery) with AS (3 mg/kg, s.c.). Mechanical (Panel A) and thermal (Panel B) hyperalgesia were daily evaluated (Panel A and Panel B, respectively). n=6 per group per experiment, representative of two experiments. *P<0.05 compared to sham group and #P<0.05 CCI + 3 mg/kg AS compared to CCI + vehicle group. Two-way ANOVA followed by Tukey's *post hoc*.

Figure 4. AS reduces CCI-induced spinal cord glial cell activation in mice. At 5 h after acute (7 days after surgery) or chronic (7-14 days after surgery) AS treatment (3 mg/kg, s.c.), spinal cord samples of mice were collected to determine the GFAP (Panel A) and Iba-1 (Panel B) mRNA expression by qPCR. n=6 per group per experiment, representative of two experiments. *P<0.05 compared to sham group and #P<0.05 compared to CCI + vehicle group. One-way ANOVA followed by Tukey's test.

Figure 5. AS reduces CCI-induced spinal cord up-regulation of pro-IL-1 β , TNF- α , IL-33 and ST2 receptor mRNA expression in mice. At 5 h after acute (7 days after surgery) or chronic (7-14 days after surgery) AS treatment (3 mg/kg, s.c.), spinal cord samples of mice were collected to determine pro-IL-1 β (Panel A), TNF- α (Panel B), IL-33 (Panel C), and ST2 receptor (Panel D) mRNA expression by qPCR. n=6 per group per experiment, representative of two experiments. *P<0.05 compared to sham group and #P<0.05 compared to CCI + vehicle group. One-way ANOVA followed by Tukey's test.

Figure 6. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine plasma levels. The blood levels of AST (Panel A), ALT (Panel B), urea (Panel C) and creatinine (Panel D) were analyzed after chronic treatment (between 7-14 days after CCI surgery). Data are presented as means $\pm$ standard error of the mean (S.E.M.) of nine animals per group. No statistical differences were observed. One-way ANOVA.

TABLE

Table 1: Primer sequences for PCR.

<i>Gene</i>	<i>Forward</i>	<i>Reverse</i>
<i>Gadph</i>	5' CATACCAGGAAATGAGCTTG 3'	5' ATGACATCAAGAAGGTGGTG 3'
<i>GFAP</i>	5' GGCGCTCAATGCTGGCTTCA 3'	5' TCTGCCTCCAGCCTCAGGTT 3'
<i>Iba1</i>	5' ATGGAGTTTGATCTGAATGGAAAT 3'	5' TCAGGGCAGCTCGGAGATAGCTTT 3'
<i>Pro-IL-1β</i>	5' GAAATGCCACCTTTTGACAGTG 3'	5' TGGATGCTCTCATCAGGACAG 3'
<i>TNF-α</i>	5' TCTCATCAGTTCTATGGCCC 3'	5' GGGAGTAGACAAGGTACAAC 3'
<i>IL-33</i>	5' TCCTTGCTTGGCAGTATCCA 3'	5' TGCTCAATGTGTCAACAGACG 3'
<i>ST2</i>	5' GCAATTCTGACACTTCCCATG 3'	5' ACGATTTACTGCCCTCCGTA 3'

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

Highlights

- Angeli's salt (AS) diminished CCI-induced neuropathic pain in mice
- AS analgesia depends on the activating the cGMP/PKG/K⁺ATP signaling
- Acute AS treatment inhibited CCI-induced Iba1, IL-1 β and ST2 spinal cord expression
- Chronic AS treatment inhibited CCI-induced spinal cord GFAP and Iba-1 expression
- Chronic AS inhibited CCI-induced spinal cord IL-1 β , TNF- α and IL-33/ST2 expression