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Research Paper

Pre-emptive intrathecal quinidine alleviates spinal nerve ligation-induced peripheral neuropathic pain

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Abstract

Objectives Quinidine, a class I anti-arrhythmic agent, is a sodium channel blocker that is more potent than lidocaine and mexiletine. This study tested pre-emptive intrathecal quinidine to attenuate neuropathic pain induced by lumbar spinal nerve ligation (SNL).

Methods Ninety-six adult male Sprague–Dawley rats were grouped equally ($n = 24$ per group) as follows: group S (sham), removal of transverse process only; group L, SNL; group Q_{35}, SNL pretreated with intrathecal quinidine 35 mM (50 μl); group Q_{70}, SNL pretreated with intrathecal quinidine 70 mM (50 μl). Neuropathic pain was measured by thermal hyperalgesia and mechanical allodynia. Other measurements included dys-regulation of sodium channel $Nav_{1.3}$ in dorsal root ganglion (DRG) and spinal microglia activation in spinal dorsal horn.

Key findings Spinal nerve ligation induced abnormal mechanical allodynia and thermal hyperalgesia, up-regulated $Nav_{1.3}$ in DRG, and activated microglia in spinal cord. Group Q_{70} showed attenuated thermal hyperalgesia ($P < 0.001$) and mechanical allodynia ($P < 0.05$) on postoperative day 5 (POD_5) but not on POD_7, reversed up-regulated expression of $Nav_{1.3}$ on POD_3 and POD_7 in DRG and significantly attenuated microglia activation on POD_7 ($P = 0.032$) in spinal cord.

Conclusions Pretreatment with intrathecal quinidine 70 mM before SNL attenuates nerve ligation-induced neuropathic pain. The duration of the effect is 5 days.

Keywords neuropathic pain; pre-emptive intrathecal quinidine; spinal nerve ligation

Introduction

Pretreatment with intraspinal opioid and local anaesthetic has been reportedly effective for reducing postoperative pain by blocking afferent nociceptive input and by reducing central nervous system sensitization from noxious stimuli.[1,2] In patients with pre-emptive analgesia, local anaesthesia can inhibit nerve impulse from noxious stimuli by deactivating voltage-gated sodium channels (VGSCs) and can also reduce the need for postoperative morphine.[3] As for pretreatment with local anaesthetics on injured nerve, localized infiltrations of lidocaine or bupivacaine are able to suppress injury-induced neuropathic pain.[4] Lidocaine administered intrathecally reverses tactile allodynia caused by nerve injury.[5,6] This demonstrates that blockade of nerve impulses with sodium channel blocker from the nerve injury site to the dorsal root ganglion (DRG) neuron cells, either before or immediately after nerve damage, prevents abnormal stimuli and effectively relieves neuropathic pain. The blockade of abnormal nerve impulses is mainly through inhibition of dys-regulated VGSCs on the injured site and on injured DRG.[7] Among the dys-regulated VGSCs expressions, up-regulated $Nav_{1.3}$ occurred in a subpopulation of DRG neurons after nerve injury.[8] Furthermore, impulses of noxious stimuli from injured nerve transmitted upward to spinal dorsal horn provoked microglia overactivity and aggravation of

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neuropathic pain.[9] However, pre-emptive intrathecal administration of sodium channel blocker has not been tested for effectiveness in controlling neuropathic pain.

Quinidine, a class I anti-arrhythmic sodium channel blocking agent, not only provides local anaesthesia by blocking the fast inward sodium current,[10] but also has multiple effects on both inward and outward potassium currents.[7] Compared with lidocaine or mexiletine, quinidine has greater potency as a sodium channel blocker for its anti-arrhythmic[11] and cutaneous analgesic effects.[12] For managing neuropathic pain, blockade of VGSCs by others class I anti-arrhythmic agents, such as lidocaine, mexiletine and flecainide, has proven effective in chronic pain patients.[13] Until now, however, the use of quinidine for treating chronic pain has been limited to the inhibiting of cytochrome P450-2D6 rather than the blocking of VGSCs.[14] In this study, neurobehavioral examinations were performed to assess whether intrathecal quinidine pretreatment reduces neuropathic pain. Its potential therapeutic effects were assessed by measuring dys-regulated $Nav_{1.3}$ in primary neuron cells in the DRG[15] and activation of spinal microglia in spinal dorsal horn.

Materials and Methods

Ninety-six adult male Sprague–Dawley rats, 300–350 g, were used. Ethical approval for this study (Approval no. 96130) was provided by the Institutional Animal Care and Use committee of Kaohsiung Medical University, Kaohsiung, Taiwan (chairperson Fa Po Chung) on 15 November 2008. Rats were allocated into four groups ($n = 24$ in each group): sham group (group S), removal of left sixth lumbar spine transverse process; ligation group (group L), surgery with spinal nerve ligation (SNL) only; intrathecal quinidine 70 mM group (group Q_{70}), SNL and pretreatment with intrathecal quinidine 70 mM; intrathecal quinidine 35 mM group (group Q_{35}), SNL and pretreatment with intrathecal quinidine 35 mM. Behavioral testing to noxious thermal stimuli and to mechanical stimuli applied to the hindpaws were performed on pre-operative (pre-OP) day 1 and on postoperative days 3, 5 and 7 (POD_3, POD_5, POD_7) and then weekly thereafter for the next 7 weeks (POD_{14}, POD_{21}, POD_{28}, POD_{35}, POD_{42}, POD_{49} and POD_{56}).

After local lidocaine anaesthesia, quinidine 50 µl (70 mM or 35 mM) was administered by intrathecal injection through the $L_{4\text{-}5}$ intervertebral space. Intrathecal quinidine was considered successful if rats were observed dragging their hind limbs during movement[16] and application of Adson forceps on lateral paws did not elicit painful withdrawal response (it takes about 2–3 min). After then, under isoflurane/O_2 anaesthesia, the L_5 spinal nerve was isolated and tightly ligated with a 6–0 Dexon in SNL groups within 15 min. Surgical procedures were identical in other group except ligation of the left L5 spinal nerve was not performed in group S.

For neurobehavioral examination, the latency of foot withdrawal from noxious heat stimuli was measured using the method described by Kim and Chung.[17] The time from application of light beam to lifting of hind paw was recorded and was defined as foot withdrawal latency. Mechanical allodynia to calibrate von Frey filaments (Stoelting, Wood Dale, USA) applied to the plantar surface of the hindpaw were also measured.

For Western blotting, under thiopentone anaesthesia, the left L_5 DRGs were removed ($n = 8$ from each group, 4 on POD_3 and 4 on POD_7) and extracted. Fifty micrograms of total protein was fractionated on 8% sodium dodecyl sulfate–polyacrylamide gels (SDS-PAGE) and blotted on polyvinylidene fluoride membranes (PVDF, Millipore Corp., MA, USA). Primary antibodies (rabbit anti-$Nav_{1.3}$; Alomone Labs, Jerusalem, Israel) were detected with horseradish peroxidase-conjugated mouse anti-rabbit antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, USA). The intensity of each band was expressed by ECL Western blotting detection reagents (Amersham Biosciences, Uppsala, Sweden). The bands of $Nav_{1.3}$ were measured and normalized with the corresponding loading control, β-actin. The relative values of $Nav_{1.3}$ in the sham group and experimental groups were expressed as ratios.

Spinal microglia activation in L_5 spinal dorsal horn after SNL were evaluated by immunofluorescence. Under thiopentone anaesthesia and 4% paraformaldehyde perfusion, the L_5 spinal cord tissues from each group ($n = 8$, 4 on POD_7 and 4 on POD_{14}) were sampled. Dissected tissues were fixed in 4% paraformaldehyde then saturated in 10–30% sucrose. The 12 µm of spinal cords tissues were cryostat cut and mounted onto glass slides for immunostaining. To express activation of microglia in spinal cord, mouse monoclonal anti-OX-42 (mouse anti-rat-cd 11, 1 : 80; BD Biosciences, San Jose, CA, USA) and goat anti-mouse IgG Alexa Fluor 488 (Invitrogen Life Technologies, Paisley, UK) secondary antibody were

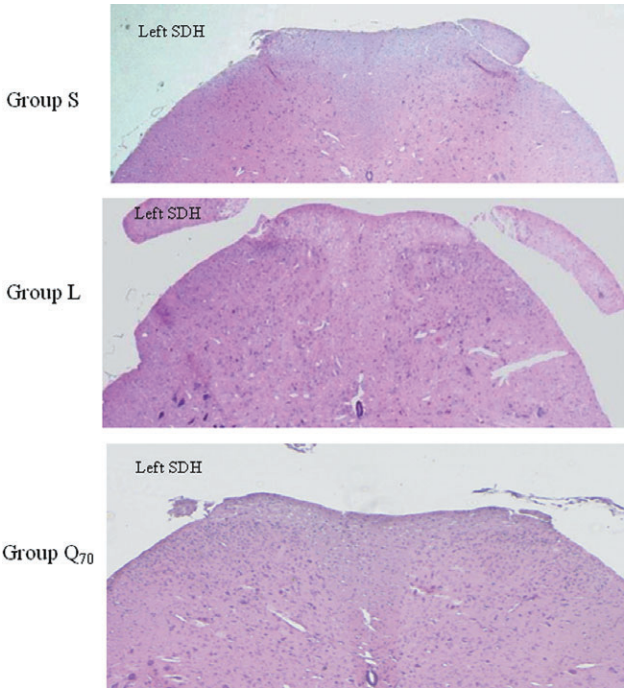

Figure 1 Histological staining of rat spinal cord. Increased microglia cells were observed on the dorsal horns of group L and group Q_{70} but not on those of group S. White matter revealed no obvious demyelination or extended inflammatory cell infiltration. SDH, spinal dorsal horn.

used. To detect the activation of microglia from p-p38 MAP kinase expression, the primary antibody p-p38 MAP kinase (1 : 200; Cell Signaling, Beverly, USA) and Cy3-conjugated goat anti-rabbit secondary antibody (Chemicon, International, Temecula, CA, USA) were used. For double immunofluorescence labelling, sections were incubated for 48 h at 4°C with a mixture of p-p38 MAP kinase antibody (1 : 100) and mouse monoclonal anti-OX-42 antibody. The Cy3 or Alexa Fluor 488-conjugated secondary antibodies were used to bind the primary antibodies. Background immunostaining was performed in the absence of primary antibodies. The stained sections were examined and images were captured using an Olympus LSM_5 fluorescence microscope. Quantification of immunofluorescence staining in the spinal cord was performed by means of a computerized imaging system to analyse positive staining for microglia or p-p38 on the ipsilateral side of the dorsal horn of the spinal cord. Background fluorescence intensity of each tissue section was subtracted. Six sections were evaluated in each rat; four rats were used for statistical analysis and the average density of microglia and p-p38 in each group was obtained.

For histological staining, formalin-fixed spinal cord specimens were sliced to a thicknesses of 3~4 μm, stained with hematoxylin and eosin (H&E), then viewed under a microscope.

Statistical analysis

Group differences were compared by analysis of variance followed by multiple post-ho*c* analyses in Western blotting, microglia and p-p38 MAP kinase activation. Behavioral data, were analysed by Mann–Whitney *U*-test.

Results

Ipsilateral (left) and contralateral (right) behaviours were similar in sham rats; in all tests, left and right hind paws did not significantly differ in withdrawal latency from mechanical allodynia and thermal hyperalgesia. Comparing group L with group Q_{70} on histological staining of spinal dorsal horn, pretreatment with intrathecal quinidine did cause demyelination or extended inflammatory infiltrations of white matter (Figure 1). In thermal hyperalgesia, the left and right hindpaws did not significantly differ in withdrawal latency in

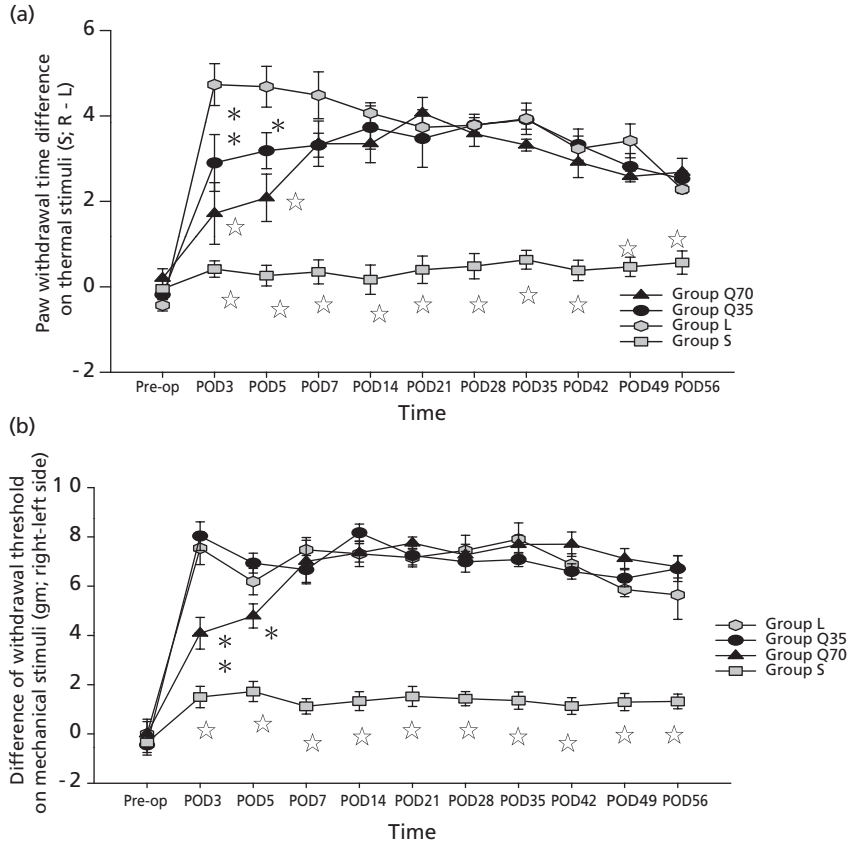

Figure 2 Behavioral testing of rats to thermal and mechanical stimuli. The difference in mean withdrawal time between the left side and the right side increased significantly in SNL groups but not in group S ($P < 0.001$). (a) Thermal hyperalgesia: SNL increased response difference between hindpaws. Pretreatment with intrathecal quinidine attenuated thermal responses on POD_3 (group L vs Q_{70}, $P < 0.001$; group L vs Q_{35}, $P < 0.01$) and POD_5 (group L vs Q_{70}, $P < 0.001$; group L vs Q_{35}, $P < 0.05$) but not on POD_7 and follow-up. (b) Mechanical allodynia: SNL increased difference of mean mechanical withdrawal threshold between hindpaws. Pre-emptive intrathecal quinidine attenuated mechanical allodynia on POD_3 (group L vs Q_{70}, $P < 0.01$) and on POD_5 (group L vs Q_{70}, $P < 0.05$) but not on POD_7 or thereafter. Values are expressed as mean ± SE. *$P < 0.05$; **$P < 0.01$; ☆$P < 0.001$ (Mann–Whitney *U*-test). s, second; gm, gram.

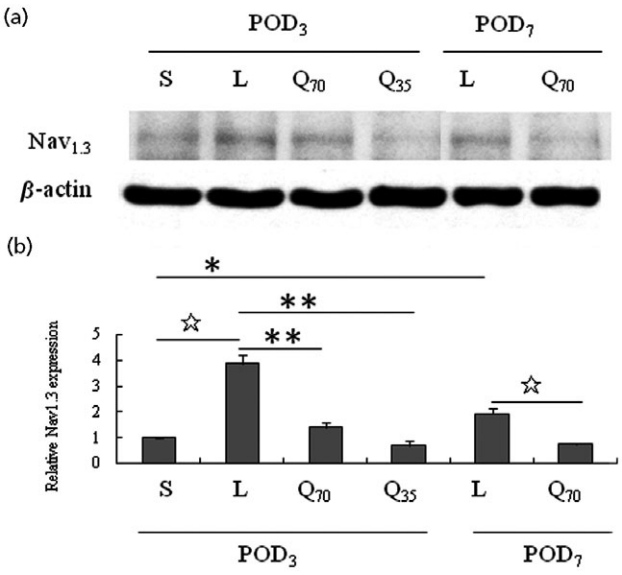

Figure 3 Increased sodium channel $Nav_{1.3}$ expression in rat L_5 injured DRG. (a,b) Increased $Nav_{1.3}$ expression on POD_3 and POD_7 in SNL groups; group Q_{70} decreased $Nav_{1.3}$ expression on POD_3 and POD_7. Each bar represents the mean ± SE. $^{\star}P < 0.001$; $^{\star\star}P < 0.01$; $^{\star}P < 0.05$ (analysis of variance followed by Scheffe test of multiple post-hoc analyses).

group S (Figure 2a). Compared with group S, all SNL groups had significantly decreased withdrawal latency ($P < 0.001$). However, in the SNL groups, pretreatment with intrathecal quinidine diminished the severity of noxious thermal responses on POD_3 (group L vs Q_{70}, $P < 0.001$; group L vs Q_{35}, $P < 0.01$) and POD_5 (group L vs Q_{70}, $P < 0.001$; group L vs Q_{35}, $P < 0.05$) but not on POD_7 and thereafter. Mechanical allodynia was noted in all nerve-ligated rats. In group Q_{70}, however, allodynia was attenuated on POD_3 ($P = 0.008$) and on POD_5 ($P = 0.04$) but not on POD_7 or thereafter (Figure 2b).

Measurements of sodium channel expression in DRG demonstrated that SNL significantly up-regulated $Nav_{1.3}$ on POD_3 and POD_7 (Figure 3). Comparing group L with groups pretreated with quinidine, group Q_{70} reversed up-regulated $Nav_{1.3}$ on POD_3 ($P = 0.004$) and POD_7 ($P < 0.001$) and group Q_{35} reversed up-regulated $Nav_{1.3}$ on POD_3 ($P = 0.001$). Microglia activation was verified by analysing OX-42 immunoreactivity in the L_5 spinal dorsal horn on POD_7 and POD_{14}. Low immunoreactivity of OX-42 microglia and p-p38 MAP kinase was observed in group S (Figures 4a and 5a). Significant microglia activation after SNL was observed in the left (ipsilateral) spinal dorsal horn on POD_7 ($P = 0.005$; Figure 4b) and POD_{14} ($P = 0.006$; Figure 4c). The immunofluorescence of p-p38 MAP kinase was significantly increased on POD_7 ($P < 0.001$) and POD_{14} ($P = 0.047$, Figure 5). Results of double immunofluorescence stains of OX-42 and p-p38 MAP kinase showed that p-p38 colocalized with microglia cells in spinal cord (Figure 5c).

Intrathecal quinidine 70 mM before SNL (group Q_{70}) attenuated OX-42 expressions of microglia on POD_7 ($P = 0.032$; Figure 4d) but not on POD_{14} ($P = 0.76$). It also reduced p-p38 MAP kinase intensity on POD_7 ($P < 0.001$; Figure 5a and 5b) but not on POD_{14} ($P = 0.71$). This indicates that quinidine 70 mM significantly decreased activated microglia and reduced p-p38 MAP kinase activity for up to one week. However, quinidine 35 mM produced no such effects (not significantly different).

Discussion

This study showed that SNL in the rat causes abnormal behavioral responses in thermal hyperalgesia and mechanical allodynia, up-regulated $Nav_{1.3}$ in injured dorsal root ganglion and increased activation of microglia cells in the spinal dorsal horn. Intrathecal quinidine before SNL reduces injured nerve-induced neuropathic pain and blunts up-regulated $Nav_{1.3}$ and activated microglia by SNL.

Tzeng *et al*.[12] previously reported that 3 μmol and 3.5 μmol doses of quinidine produced cutaneous analgesia of 88% and 100% in rats, respectively. Therefore, the indicated dose of intrathecal quinidine was chosen to be 3.5 μmol in 50 μl (70 mM) in this study. Furthermore, behavioral testing of right hindpaw and characteristics of white matter were studied to determine whether intrathecal quinidine induced spinal cord damage or neural toxicity in this study. The right hindpaw of rats recovered without abnormal behavioral responses and there was no altered Nav expressions in right DRG, no obvious increased spinal activated microglia and no increased microglia activity as compared with group S. Examination of the transverse section of the L_5 spinal dorsal horn in group Q_{70} revealed no obvious white matter demyelination and no extended infiltration of inflammatory cells. Therefore, intrathecal quinidine should not be harmful to the nerve roots and spinal cord.

The TTX-S $Nav_{1.3}$ is expressed normally only during early stages of development and is virtually undetectable in the adult rat nervous system.[18] After peripheral axotomy, $Nav_{1.3}$ expression increased in injured DRG neurons and there was reduced threshold of action potential and/or a relatively high firing frequency.[19] These axotomized DRG neurons with increased $Nav_{1.3}$ expression alter the threshold of overshooting action potentials to hyperpolarize and require less depolarization for activation.[20] Although ectopic activity abruptly increases soon after injury, it significantly diminishes within one week.[21] The $Nav_{1.3}$ expression on the lumbar spinal dorsal horn is associated with development of central neuropathic pain.[8] However, in primary sensory neurons, the role of $Nav_{1.3}$ in neuropathic pain after nerve injury is still unclear. Berta *et al*.[22] stated that functional expression of different VGSCs is altered by significantly increased $Nav_{1.3}$ and decreased $Nav_{1.8}$ channels one week after SNL. The imbalance of substantially reduced TTX-R ($Nav_{1.8}$ and $Nav_{1.9}$) current densities and the slight increase in TTX-S ($Nav_{1.3}$, $Nav_{1.6}$ and $Nav_{1.7}$) current densities cause the recovery rate to change from inactive to accelerate. The increased $Nav_{1.3}$ expression after SNL suggests that the preserved TTX-S currents and accelerated repriming are at least partly due to up-regulated $Nav_{1.3}$. However, Lindia *et al*.[23] reported that intrathecal antisense oligonucleotide administration five days after chronic constriction injury reduces $Nav_{1.3}$ expression in the axotomized DRG neurons by 50% but does not attenuate mechanical or cold allodynia. Therefore, complex changes in electrogenesis after nerve injury include not only those of the $Nav_{1.3}$ but also those of $Nav_{1.6}$ and $Nav_{1.7}$. Intrathecal quinidine

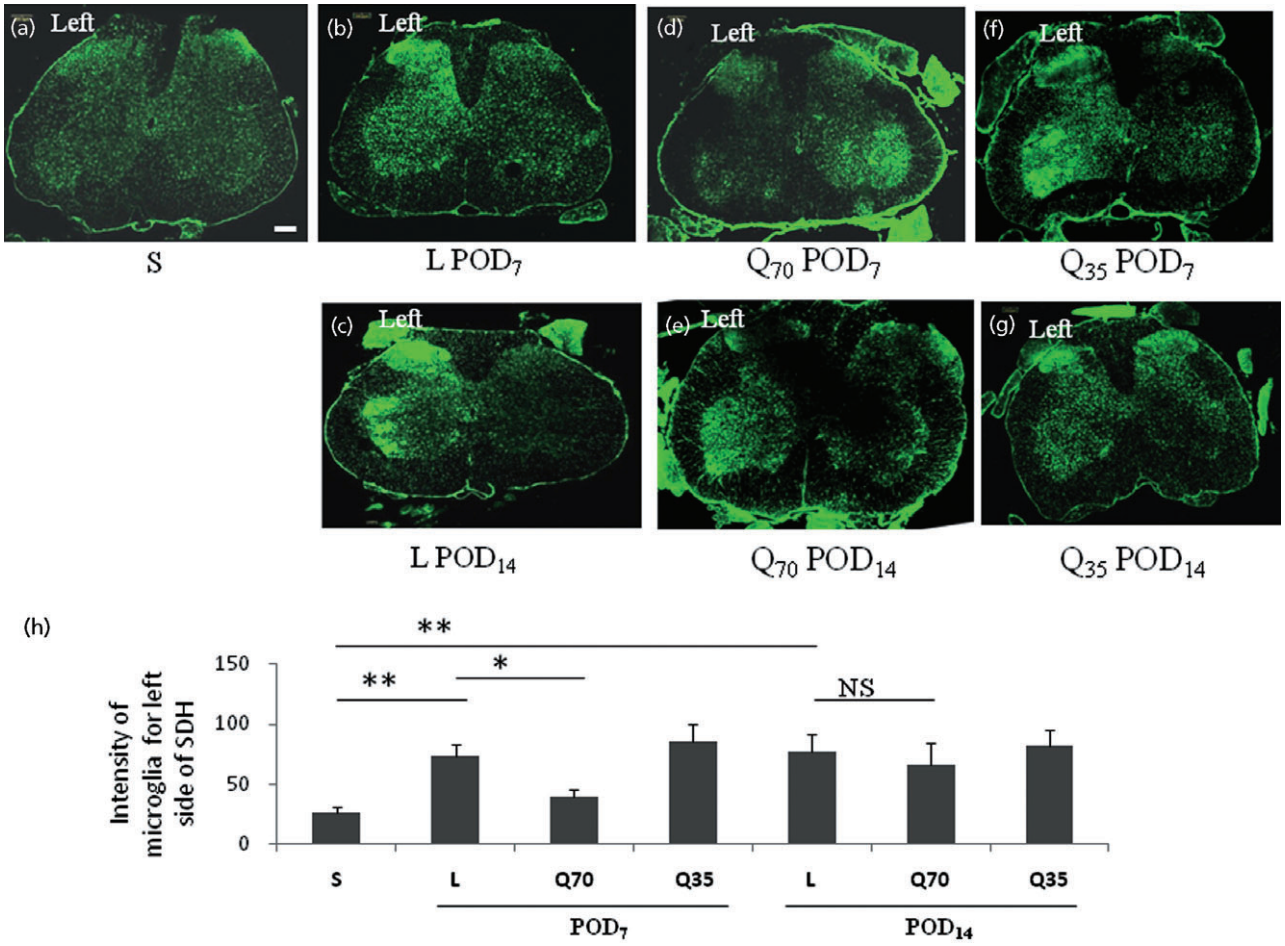

Figure 4 Microglia OX-42 expressions in transverse section of rat L_5 spinal cord. Low immunoreactivity of OX-42 microglia was observed in group S. (a) Increased expression of group L on POD_7 and POD_{14}. (b,c). Pre-emptive intrathecal quinidine 70 mM (Q_{70}) decreased intensity of immunostaining on POD_7 (d) but not on POD_{14} (e). Quantification of microglia immunoreactivity in L_5 spinal dorsal horn (h). Scale bar = 200 μm. NS, not significantly different. SDH, spinal dorsal horn. Each bar represents the mean ± SE. $^{**}P < 0.01$, $^{*}P < 0.05$ (analysis of variance followed by Scheffe test of multiple post-hoc analyses).

blocks all VGSCs. Since $Nav_{1.3}$ upregulation is blunted by intrathecal quinidine in the axotomized DRG neurons, we speculated that it may also blunt the alteration of neuronal electrogenesis in the $Nav_{1.6}$ and $Nav_{1.7}$. However, further study is needed to confirm this.

Upregulation of $Nav_{1.3}$ in injured DRG produces high frequency firing to spinal dorsal horn,[19] activates second-order neurons in spinal dorsal horn[24] and then from activated neurons to indirectly activated resting microglia and increases phosphorylation of p-38 MAP in spinal cord.[9] Spinal activated microglia play a major role in generating neuropathic pain.[9,25] Microglia activation apparently depends on dorsal root ganglion-mediated signals to the dorsal horn.[26] These abnormal impulses transmitted into dorsal horn induced not only spinal microglia hypertrophy but also increased microglia activity by way of phosphorylation of MAP kinase.[9] Acute peripheral mechanical allodynia is also associated with phosphorylation of p38 modulation of tetrodotoxin-resistant sodium channels in DRG neurons.[27] In this study, pre-emptive intrathecal quinidine 70 mM (group Q_{70}) blunted spinal activated microglia expression on POD_7 but neuropathic pain was not attenuated on POD_7. This means that inadequate reduction of activated microglia did not decrease SNL-induced neuropathic pain.

Pre-emptive intrathecal local anaesthetic before nerve damage mimicks regional anaesthesia before surgery in routine clinical procedures. Whether a single dose of pre-emptive intrathecal lidocaine or bupivacaine helps to relieve postoperative pain or neuropathic pain has not been determined.[28,29] In this study, pre-emptive intrathecal quinidine temporarily, but not totally, alleviated abnormal behavioral responses. This hints at the limitation of pre-emptive intrathecal quinidine and other sodium channel blockers, such as lidocaine and bupivacaine, may have a similar trend. Therefore, increasing the dose before injury and post-lesion quinidine administration should be the next steps to be carried out.

Conclusion

Pre-emptive intrathecal quinidine, a sodium channel blocker, attenuated thermal hyperalgesia and mechanical allodynia

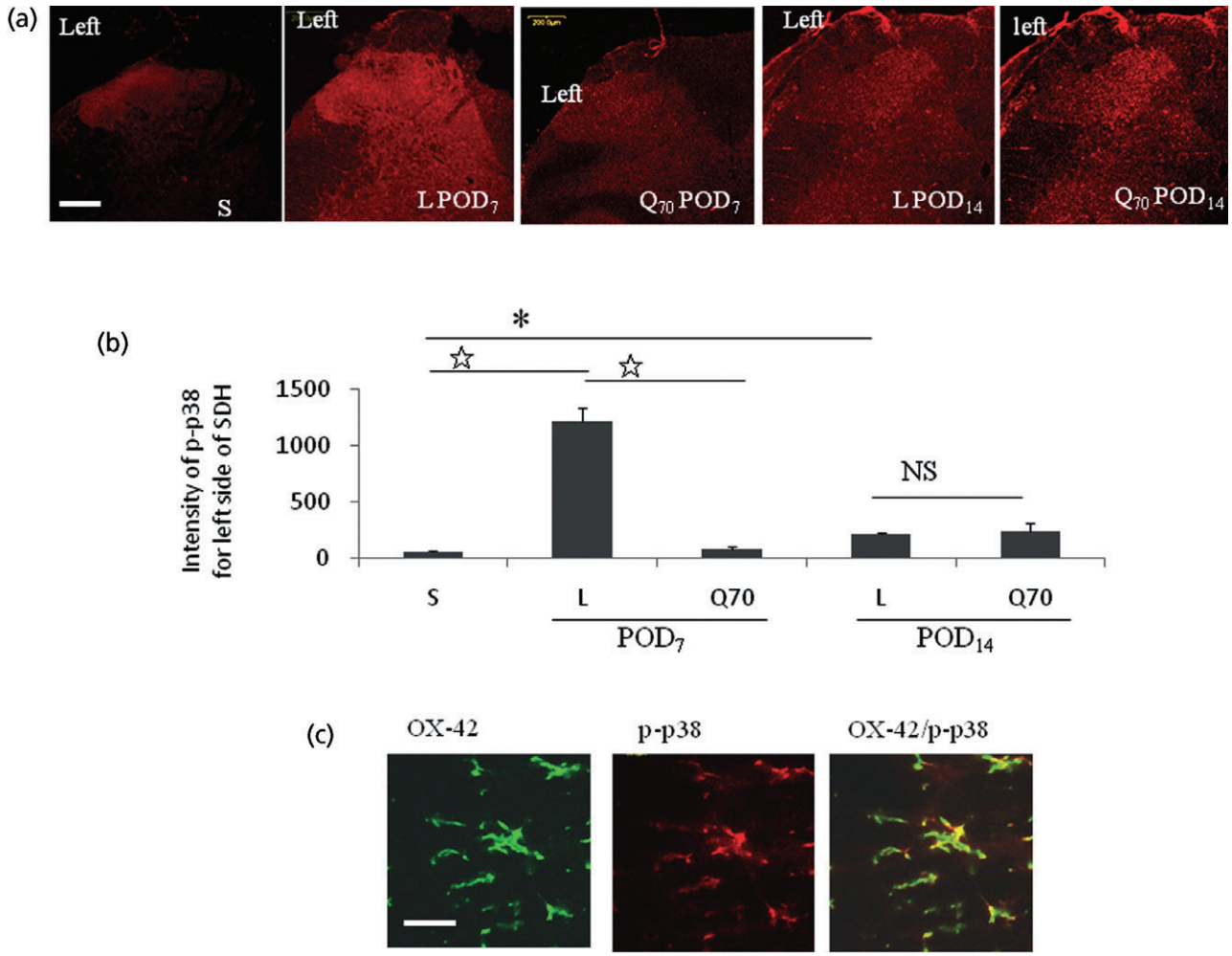

Figure 5 Phosphorylation of p38 (P-p38) mitogen-activated protein (MAP) kinase expressions on rat L_5 spinal dorsal horn. (a) Spinal nerve ligation increased p-p38 MAP kinase expression in spinal dorsal horn on POD_7 and POD_{14}. Pre-emptive intrathecal quinidine 70 mM (Q_{70}) decreased activity of p-p38 kinase on POD_7 but not on POD_{14}. (b) Intensity of p-p38 immunostaining was quantified in dorsal horn of ipsilateral spinal cord. (c) Double immunofluorescence stains on left spinal cord on POD_7. OX-42 and p-p38 MAP kinase were merged. Results showed that p-p38 colocalized with microglia cells. Scale bar = 200 μm (a); 20 μm (c). NS, not significantly different; SDH, spinal dorsal horn. Each bar represents the mean ± SE. ☆$P < 0.001$, *$P < 0.05$ (analysis of variance followed by the least significant test of multiple post-hoc analyses).

induced by lumbar spinal nerve ligation, blocked SNL-induced aberrant up-regulation of $Nav_{1.3}$ in injured DRG and reduced spinal activated microglia and phosphorylated p-p38 kinase. However, the effects of pre-emptive intrathecal quinidine are not persistent, lasting no longer than one week.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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