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Original Research

Laser irradiation activates spinal adenosine A1 receptor to alleviate osteoarthritis pain in monosodium iodoacetate injected rats

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The analgesic role of the adenosine A1 receptor is thought to involve the modulation of the spinal N-methyl D-aspartate receptor-mediated nociceptive pathway, which is suggested to be an underlying mechanism in chronic pain. Knee osteoarthritis is a degenerative condition accompanied by chronic pain. We have demonstrated that 10.6-μm laser irradiation has an antinociceptive effect in the monosodium iodoacetate -induced knee osteoarthritis in rats. However, its mechanism of action has yet to be explored. In the present work, we investigate the mechanism of 10.6-μm laser irradiation mediated antinociception in the monosodium iodoacetate -induced knee osteoarthritis. Results showed that the 10.6-μm laser significantly reversed the monosodium iodoacetate -induced nociceptive behaviors for up to 28 days. Moreover, the up-regulation of the A1 receptor and the down-regulated phosphorylation of the N-methyl D-aspartate receptor 1 subunit of the N-methyl D-aspartate receptor were observed in the spinal cord dorsal horn in the monosodium iodoacetate injected rats treated by laser irradiation. Intrathecal injection of 8-cyclopentyl-1,3-dipropylxanthine markedly reversed the effects of laser irradiation, as evidenced both by behavioral pain tests and by levels of spinal phosphorylation of N-methyl D-aspartate receptor 1. These results suggest that the spinal A1 receptor contributes to the antinociceptive effects of 10.6-μm laser, at least in part by inhibiting phosphorylation of N-methyl D-aspartate receptor 1 in the monosodium iodoacetate -induced knee osteoarthritis pain.

Keywords

Laser irradiation; osteoarthritis; analgesia; A1R; p-NR1; rat model

1. Introduction

Knee osteoarthritis (OA) is a chronic disease comprising degenerative changes of the affected joint accompanied by chronic pain, which can significantly affect patients' quality of daily life (Peat et al., 2001). Currently, there are no effective therapies for the long-term management of chronic joint pain. In addition to their adverse effects, nonsteroidal anti-inflammatory drugs (NSAIDs), commonly prescribed for knee OA pain, are more effective for acute pain than persistent pain (Dieppe and Lohmander, 2005). There are, however, several studies suggesting that low-intensity laser irradiation on the corresponding acupoints significantly relieved the pain experienced by patients with knee OA (Al Rashoud et al., 2014; Helianthi et al., 2016; Mohammed et al., 2018). Notably, our team has developed a laser device of 10.6-μm wavelength, which has demonstrated a thermal effect at acupoints leading to significant alleviation of pain and improvement in the joint function of patients with knee OA (Lin et al., 2020; Shen et al., 2008; Wu et al., 2012; Zhao et al., 2010). Moreover, our preclinical studies have shown the antinociceptive effect of 10.6-μm laser in the monosodium iodoacetate (MIA)-induced knee OA model in rats, which has been widely used in small laboratory animals and the assessment of OA pain. However, it does not fully mirror human OA. Nevertheless, the mechanism of antinociception of 10.6-μm laser remains unclear.

Adenosine is an important neuromodulator that modulates cellular function and neurotransmission via corresponding receptor binding (Sawynok, 2016). The adenosine A1 receptor (A1R) is expressed in both the peripheral and central nervous systems, particularly on intrinsic neurons in the spinal cord dorsal horn (SCDH) (Sawynok and Liu, 2003). Activation of A1R has been suggested as an analgesic target for pain relief (Zylka, 2011). Mechanism studies have validated the role of A1R in modulating the homeostasis of the central synaptic transmission through the potentiation of inhibitory neurotransmitters and inhibition of excitatory neurotransmitters in the superficial neurons of the SCDH, leading to decreased transmission of pain sensation (Lao et al., 2001; Li and Eisenach, 2005; Nguyen et al., 2017). This enhanced spinal inhibition may represent an underlying contributor to A1R-mediated antinociception.

A1R has also been implicated indirectly in the modulation of the spinal N-methyl D-aspartate receptor (NMDAR)-mediated nociceptive signal (Bai et al., 2017; Li and Eisenach, 2005). One possible mechanism by which this is achieved is via the downregulation of phosphorylation of NMDAR1 (p-NR1), an important subunit of NMDAR that has been shown to modulate the activation of NMDAR and promote the nociceptive transmission in several pain models (Gao et al., 2005; Zou et al., 2004). However, the interaction between A1R and N-methyl D-aspartate receptor 1 (NR1) at the spinal level has not been reported. Our previous study demonstrated that 10.6-μm laser irradiation at the ST35 (Dubi) acupuncture point significantly reversed MIA-induced knee OA pain (Li et al., 2020; Wu et al., 2014).

Goldman et al. (2010) demonstrated that A1R mediated the local antinociceptive effects of acupuncture on the inflammatory pain model, there has been considerable interest in the study of adenosine and its receptors in the mechanisms of acupuncture analgesia (Goldman et al., 2010). While the half-life of adenosine is known to be short, Hurt and Zylka (2012) generated long-lasting analgesic effects by injection of prostatic acid phosphatase before acupuncture, which led to sustained A1R concentrations and hence analgesic effects lasting up to six days. This work also highlighted the role of A1R in conferring the local analgesic effects of acupuncture (Hurt and Zylka, 2012; Zylka et al., 2008). However, there are outstanding controversies regarding the role of spinal A1R in acupuncture or electroacupuncture (EA)-mediated analgesia in the central nervous system. For example, Zhang et al. (2018) found that intrathecal injection of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) reversed the analgesic effects of EA at ST36 (Zusanli) and LR3 (Taichong) in a neuropathic pain model via the suppression of both astrocyte activation and tumor necrosis factor-α (TNF-α) upregulation. Conversely, Jiang et al. (2018) observed that A1R was unchanged when EA was applied along the spine, bilaterally in the same neuropathic pain model.

2. Materials and methods

2.1 Animals

Sprague-Dawley (SD) male rats (Xipuer-Bikey Co., Ltd., Shanghai, P. R. China. SCXK2013-0016) weighing 220-250 g were housed under a controlled condition (22-24 °C, relative humidity of 40-60%, and 12/12 h light-dark cycle). They were free to the food and water and were acclimatized to this environment for at least one week before experimentation.

2.2 Grouping and design

To explore the antinociceptive effects of 10.6-μm laser treatment, rats were randomly distributed into three groups: saline group, laser group, and sham control group (n = 8 per group). Using another set of rats, we specified the role of A1R in the analgesic effects of 10.6-μm laser irradiation using a selective A1R antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Sigma-Aldrich, USA), as well as dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) as solvent control. These second set of rats were assigned randomly to three groups: saline group, MIA + Laser + DPCPX group, and MIA + Laser + DMSO group (n = 8 per group).

Pain behavioral tests were conducted on day 1 (baseline) before MIA injection and on days 3, 7, 14, 21, and 28 post-MIA injection. Laser irradiation was administered daily on day 1 to day 7 post-MIA injection. DPCPX was administered intrathecally 1h before behavioral pain tests on day 28.

2.3 004DIA-induced knee OA model

Rats were anesthetized with isoflurane before MIA (3 mg/50 μl, Sigma-Aldrich, USA) was dissolved in sterile 0.9% saline and injected into the left knee joint through the infra-patellar ligament using a 26G needle. Rats assigned to saline groups were intra-articularly injected with 50 μl sterile 0.9% saline.

2.4 Intrathecal injection

The procedures and dosing of DPCPX were performed as previously described (Zhang et al., 2018). Rats were anesthetized with isoflurane. DPCPX was dissolved in 10% DMSO (10 μg/10 μL) and administered slowly between the L5 and L6 vertebrae using a Hamilton syringe followed by a 10 μL saline wash.

2.5 Laser irradiation

A 10.6-μm laser device (SX10-C1, Wonderful-Opto-Electrics Tech Co, Ltd., Shanghai, P. R. China) was applied as described in our previous studies (Li et al., 2020; Liu et al., 2018; Shen et al., 2019). Before the intervention, rats were acclimated to the experiment for 15 min and immobilized by the operator's hands on the platform (Fig. 1a). From day 1, after MIA injection, rats assigned to laser treatment were exposed to laser irradiation (wavelength, 10.6 μm; radiant power, 80 mW; beam area, 0.0314 cm^2; irradiance, 2.548 W/cm^2; each treatment dose, 48.01 J (1500 J/cm^2); cumulative dose, 336.07 J; Table 1) for 10 min once daily for 7 days at ST35, which is located in the depression of the lateral aspect of the infrapatellar ligament (Fig. 1b).

For the rats in the sham control group, the same immobilized procedures were performed, without laser output.

Table 1. The parameters of laser irradiation

Wavelength	10.6 μm
Radiant Power	80 mW
Beam Area	0.0314 cm^2
Irradiance	2548 mW/cm^2
Each Treatment Duration	10 min
Each Treatment Dose	48.01 J (1529 J/cm^2)
Treatment Frequency	once per day (total 7 applications)
Cumulative Dose	336.07J

2.6 Pain behavior tests

After 30 min of adaptation, an Electronic Von Frey Aesthesiometer (IITC Life Science, USA) was used to evaluate mechanical hyperalgesia. During the test, the middle part of the plantar left hind paw was vertically stimulated with a rigid probe until the occurrence of paw withdrawal or flinching, considered a positive response. The displayed force was recorded as the paw withdrawal mechanical threshold (PWMT).

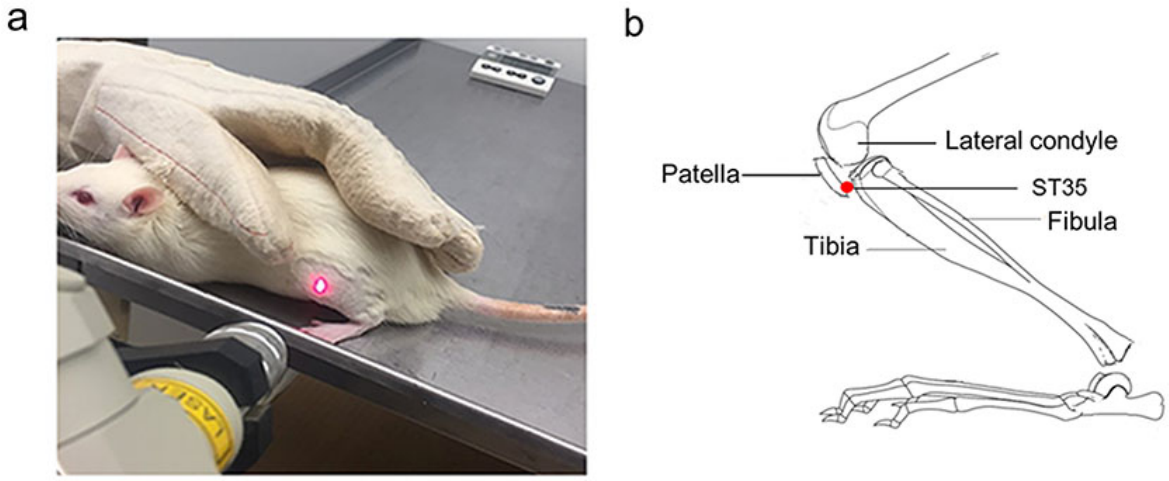

Figure 1. 10.6-μm laser irradiation on the MIA-injected rat. (a) The picture of laser irradiation at ST35 on the MIA-injected left knee joint. (b) The anatomical location of ST35. MIA, monosodium iodoacetate. ST35, the acupoint named Dubi located in the depression of the lateral aspect of the infrapatellar ligament.

The weight distribution across the left and right hind paws was evaluated by an Incapacitance Tester (MR-600, IITC Life Science). Each paw of the rat was positioned on a separate load-bearing plate, and the weight was automatically recorded. The weight distribution was calculated as: [(weight of the left hind paw)/(weight of the left + right hind paw) $\times$ 100%].

These two pain behavior tests were repeated 3 times, with an interval of 5 minutes between each test.

2.7 Western blot

Rats were euthanized after the behavioral tests on day 28 by ketamine and xylazine (100 mg/kg and 5 mg/kg, respectively, intramuscular). A lumbar segment (L4-L6) was removed, homogenized in lysis buffer, and then centrifuged at 14000 rpm/min for 15 min at 4 °C to obtain the supernatants. Bicinchoninic acid (BCA) protein assay was used to determine protein concentration. The sample was subjected to the 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. Following overnight blocking with 5% bovine serum albumin (BSA), the blots were incubated at 4 °C overnight with the following primary antibodies: anti-A1R antibody (1 : 1000; Abcam, USA), anti-NMDAR 1 (phosphor S897) antibody (1 : 1000; Abcam), and anti-GAPDH antibody (1 : 1000; Abcam), and then a horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature (RT) for 2 h. The enhanced chemiluminescence (ECL) was used to visualize the blots, and Image-Pro Plus 6.0 (Media Cybernetics, USA) was used to analyze the density of the specific bands.

2.8 Immunofluorescence

After abdominal anesthesia with sodium pentobarbital (60 mg/kg, intraperitoneal), rats underwent cardiac perfusion with 0.9% saline, followed by 4% polyformaldehyde (PFA). The removed L4-L6 spinal segment was post-fixed for another 6 h in 4% PFA and dehydrated in 30% sucrose for 48 h at 4 °C. The spinal tissues were cut into a thickness of 30 μm on a cryostat (Leica, RM2016, Germany). After blocking with 5% normal goat serum (NGS), the slices were incubated at 4 °C overnight with the following primary antibodies: anti-A1R antibody (1 : 500; Abcam), anti-NMDAR 1 (phosphor S897) antibody (1 : 500; Abcam), anti-NMDAR 1 antibody (1 : 500; Abcam), anti-Iba-1 antibody (1 : 500; Abcam), anti-GFAP antibody (1 : 500; Abcam), anti-NeuN antibody (1 : 500; Abcam) and subsequently stained with corresponding secondary antibodies to Alexa Fluor 594 or 488 (1 : 500; Abcam) for 2 h at RT. A confocal fluorescence microscope (Eclipse C1, Nikon, Japan) was applied to capture the slices, and the fluorescence intensity of each protein was analyzed by Image-Pro Plus 6.0 software.

2.9 Statistical analysis

All data were expressed as mean $\pm$ SEM and analyzed with GraphPad Prism 7.0 software (GraphPad Software, USA). Two-way ANOVA for repeated measurements followed by Tukey's post hoc test was used for pain behavior tests. For Western blot and immunofluorescence staining analysis, one-way ANOVA was performed, followed by Tukey's post hoc test for comparisons among groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 10.6-μm laser irradiation reversed the MIA-induced nociceptive behaviors in rats

MIA-injected rats presented with striking mechanical hyperalgesia and weight-bearing asymmetry up to 28 days, compared to saline-injected rats (Fig. 2a and Fig. 2b, $P < 0.001$). Compared to the sham control group, laser irradiation at ST35 significantly alleviate PWMT and a weight-bearing difference in the knee OA rats (Fig. 2a and Fig. 2b, $P < 0.05$ or $P < 0.01$). The analgesic effect of laser irradiation was evident, especially at the later stage of the 28-day experimental period, suggesting that 10.6-μm laser irradiation may confer a cumulative therapeutic effect in MIA-induced OA pain.

Besides, given the error bars of the results of pain behavior tests are too small for n = 8, several factors may be involved. The operator was trained and conducted the pain behavior tests for nearly one year in our pilot study. We performed the pain behavior tests in a closed, noiseless room with the same operator at the same

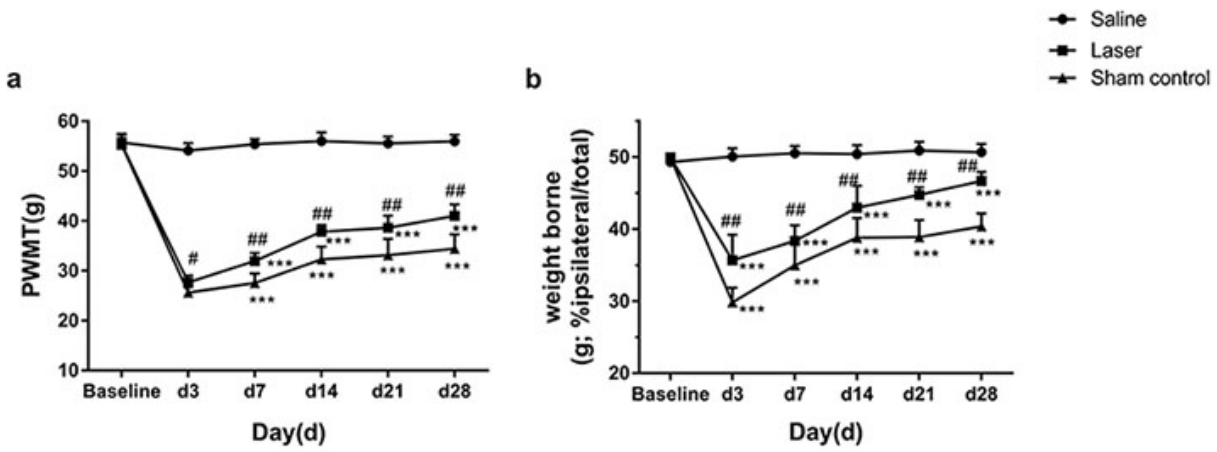

Figure 2. Comparison of MIA-induced nociceptive behaviors after 10.6 μm laser irradiation. (a) The PWMT of each rat in all groups. (b) The weight-bearing distribution of each rat in all groups. $^{***}P < 0.001$ compared to saline group; $^{\#\#}P < 0.01$ compared to sham control group, n = 8 per group. MIA, monosodium iodoacetate; PWMT, paw withdrawal mechanical threshold.

time and another assistant recording the results (both of them were blinded the grouping). Before the formal experiment, each rat was subjected to at least one week of adaptation in the testing environment and also acclimatized in the testing container for 30min per day (total of seven days) to ensure baseline comparability and reduce variability.

3.2 10.6-μm laser irradiation upregulated spinal A1R and downregulated p-NR1 in the MIA-induced knee OA model in rats

According to Western blot analysis, a reduction of A1R was observed in the sham control group relative to the saline group (Fig. 3a, $P < 0.05$), suggesting MIA induced A1R reduction. In contrast, A1R was significantly increased in the laser group compared to the sham control group and saline group (Fig. 3a, $P < 0.05$, or $P < 0.01$). Similar results were obtained with immunofluorescence staining, where the fluorescence intensity of A1R in the ipsilateral SCDH was increased in the laser group relative to the sham control group and saline group (Fig. 3b, $P < 0.01$ or $P < 0.001$).

Furthermore, both protein and fluorescence intensity of spinal p-NR1 was higher in the MIA-injected rats than in the saline-injected rats (Fig. 3c and Fig. 3d, $P < 0.05$ and $P < 0.001$), indicating that MIA induced the upregulation of p-NR1. Conversely, p-NR1 in the laser group was significantly decreased compared to the sham control group (Fig. 3c and Fig. 3d, $P < 0.01$). These results suggest that 10.6-μm laser irradiation increased spinal A1R expression and decreased p-NR1 in MIA-injected rats.

3.3 DPCPX reversed the antinociceptive behavioral response of laser irradiation in MIA-injected rats

We further investigated whether A1R was involved in the laser irradiation-mediated antinociceptive effects. On day 28, before DPCPX injection, there was no statistical difference in PWMT or weight-bearing distribution in the MIA-injected rats given laser irradiation (Fig. 4a and Fig. 4b, $P > 0.05$). However, 1 h after DPCPX injection, a significantly reduced PWMT and the weight-bearing difference was observed in the MIA + Laser + DPCPX group compared with the MIA + Laser + DMSO group (Fig. 2a and Fig. 2b, $P < 0.01$), indicating that DPCPX may reverse the antinociceptive effects of 10.6-μm laser irradiation on the MIA-induced nociceptive behaviors.

3.4 DPCPX blocked the laser irradiation-mediated downregulation of p-NR1 in MIA-injected rats

Furthermore, we investigated the role of A1R on laser irradiation-mediated inhibition of NR1 phosphorylation. Double labeling showed that A1R was expressed in NeuN (Neuronal Nuclei)-labeled neurons, but not in Iba-1 (Ionized Calcium Binding Adaptor Molecule-1)-labeled microglia or GFAP (Glial Fibrillary Acidic Protein)-labeled astrocytes in the MIA-injected rats (Fig. 5a). We further observed A1R co-expressed with NR1 in NeuN-labeled neurons (Fig. 5b), suggesting A1R colocalization with NR1 in SCDH neurons of MIA-injected rats.

The protein levels of p-NR1 were significantly upregulated in the MIA + Laser + DPCPX group compared to the MIA + Laser + DMSO and saline groups (Fig. 5c, $P < 0.01$ or $P < 0.001$). These results indicate that DPCPX suppressed the 10.6-μm laser irradiation-mediated downregulation of p-NR1 in the knee OA rat model.

4. Discussion

In the present study, we investigated whether 10.6-μm laser irradiation at ST35 alleviates MIA-induced knee OA pain via the upregulation of spinal A1R and the downregulation of p-NR1 expression. Also, we further explored the role of spinal A1R in the analgesic effects of 10.6-μm laser irradiation at ST35 in an MIA-induced knee OA model, and we found that the laser irradiation significantly alleviated MIA-induced nociceptive behaviors in rats. Strikingly, laser therapy also upregulated the A1R and downregulated the p-NR1 in the SCDH of MIA-injected rats. Furthermore, the intrathecal injection of DPCPX reversed the antinociceptive effects of 10.6-μm laser, evidenced in both nociceptive behaviors and spinal p-NR1. These results suggest that spinal A1R contributes to the antinociceptive effects of the 10.6-μm laser irradiated at ST35 and that this could be conferred at least in part via the inhibition of p-NR1 in the knee OA rat model.

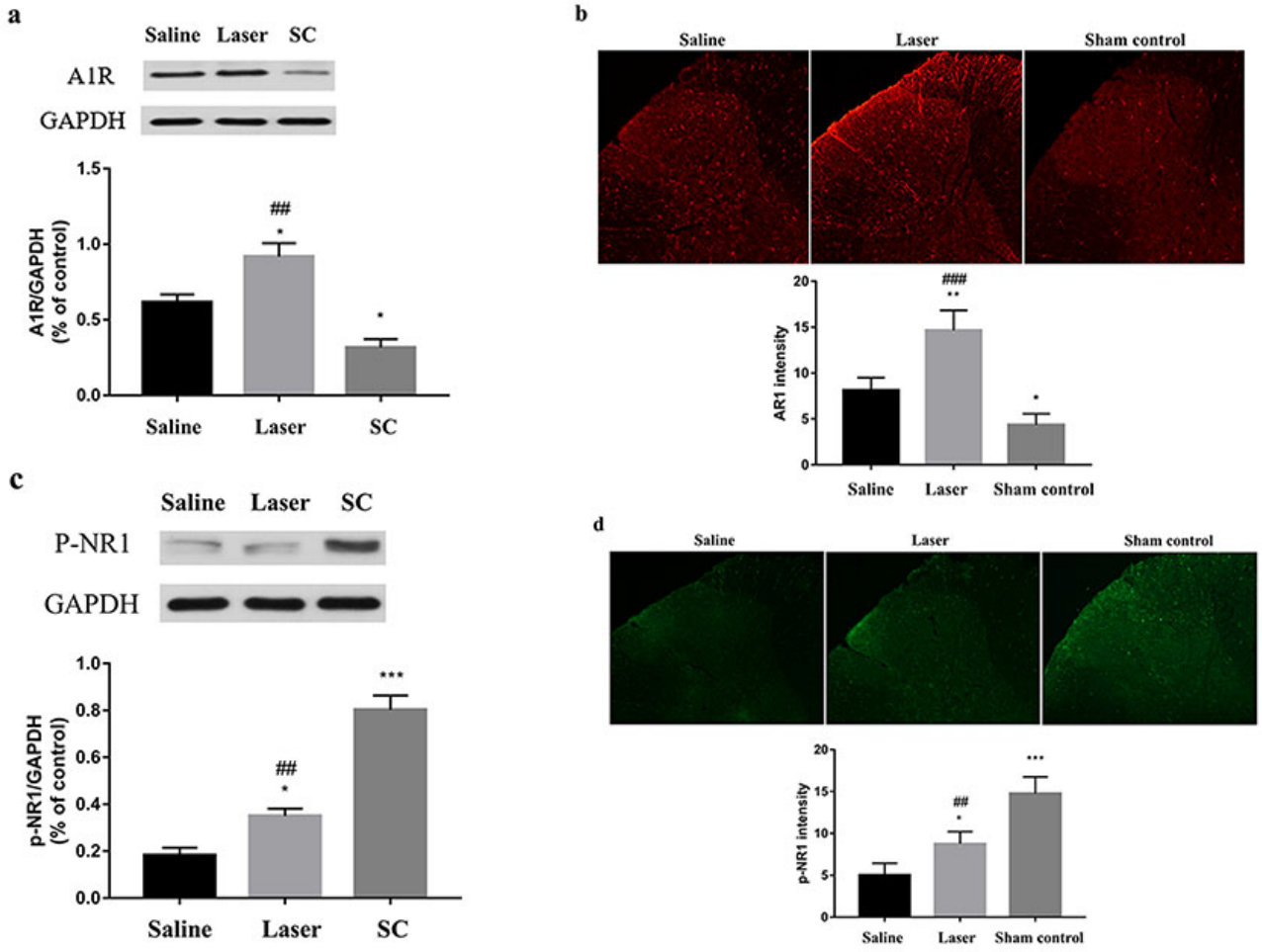

Figure 3. Comparison of spinal A1R and p-NR1 after 10.6μm laser irradiation. (a) The protein levels of spinal A1R. (b) The immunofluorescence intensity of A1R in the ipsilateral SCDH. (c) The protein levels of spinal p-NR1. (d) The immunofluorescence intensity of p-NR1 in the ipsilateral SCDH. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to saline group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to sham control group, n = 4 per group. SC, sham control, A1R, adenosine A1 receptor; p-NR1, phosphorylation of N-methyl D-aspartate receptor 1.

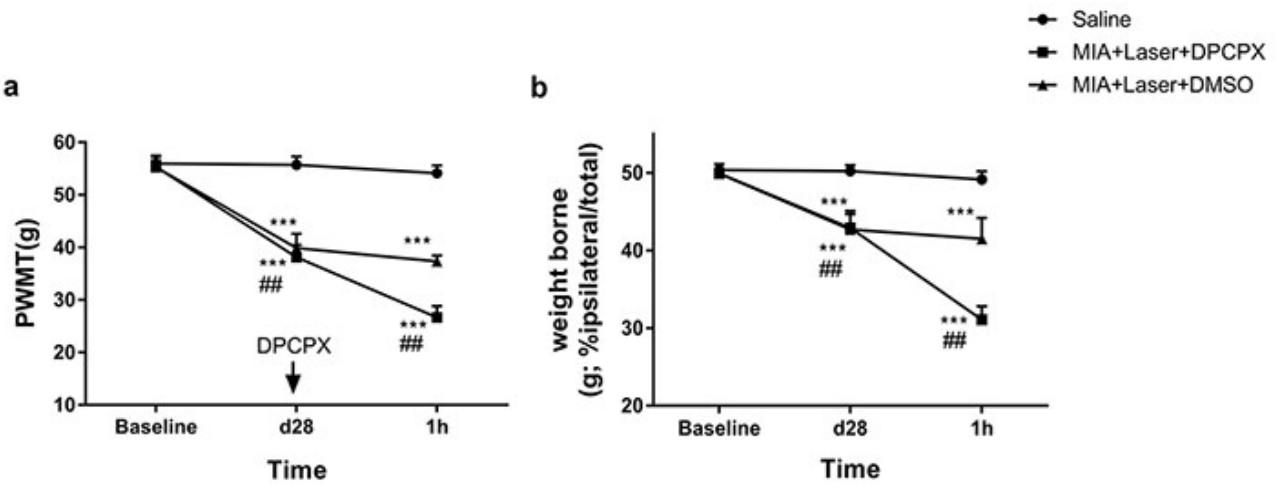

Figure 4. Comparison of MIA-induced nociceptive behaviors after drug administration. (a) The PWMT of each rat among groups. (b) The weight-bearing distribution of each rat among groups. $^{***}P < 0.001$ compared to saline group; $^{\#\#}P < 0.01$ compared to MIA + Laser + DMSO group, n = 8 per group. MIA, monosodium iodoacetate; PWMT, paw withdrawal mechanical threshold; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMSO, dimethyl sulfoxide.

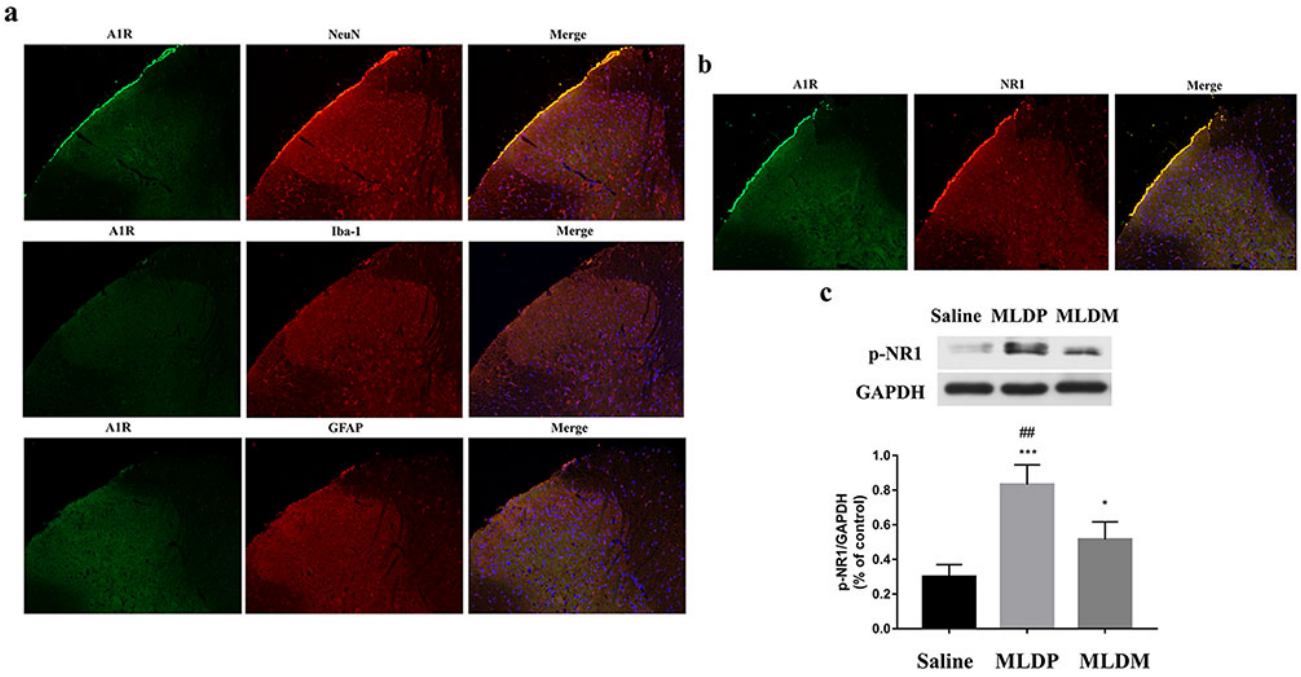

Figure 5. The distribution of A1R and NR1 in SCDH of MIA-injected rats and the comparison of spinal p-NR1 after drug administration. (a) Double immunostaining of A1R (green), and NeuN or Iba-1 or GFAP (red) in the ipsilateral SCDH. (b) Double immunostaining of A1R (green) and NR1 (red) in the neuron of ipsilateral SCDH. (c) Western blot analysis of spinal p-NR1 in MIA-injected rats. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to saline group; $^{\#\#}P < 0.01$ compared to MIA + Laser + DMSO group, n = 4 per group. MIA, monosodium iodoacetate; A1R, adenosine A1 receptor; NR1, spinal N-methyl D-aspartate receptor 1; p-NR1, phosphorylation of N-methyl D-aspartate receptor 1; SCDH, spinal cord dorsal horn; NeuN, neuronal nuclei; Iba-1, ionized calcium-binding adaptor molecule-1; GFAP, glial fibrillary acidic protein; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMSO, dimethyl sulfoxide; MLDP, MIA + Laser + DPCPX; MLDM, MIA + Laser + DMSO.

The inhibitory effects of adenosine are predominantly mediated by A1R in the nervous systems (Gong et al., 2010; Sawynok and Reid, 2012). However, the mechanisms of A1R-mediated antinociception are not fully understood. It is known that A1R is a $G_{i/o}$ protein-coupled receptor that inhibits cyclic adenosine monophosphate (cAMP)-protein kinase (PKA) signaling postsynaptically. A1R also confers presynaptic inhibition, attenuating glutamatergic release. This is thought to occur via the activation of presynaptic K^+ channels, increasing K^+ conductance and hence neuronal hyperpolarization (Patel et al., 2001), thus leading to the suppression of neurotransmitter release across the synapse (Lao et al., 2004). NMDAR, a primary excitatory neurotransmitter, is a member of the glutamate receptors, playing a critical role in synaptic plasticity and hyperalgesia (Chen et al., 2016). Among the NMDAR subunits, NR1 is well demonstrated to modulate NMDAR activity and promote the transmission of nociceptive inputs during pain procession (Gao et al., 2005; Zou et al., 2004).

Importantly, A1R has been shown to colocalize with glutamatergic neurons in the striatum, where the concentration of adenosine was also found to be regulated by NMDAR (Quarta et al., 2004). This suggests a possible interaction between A1R and NMDAR. In addition to its attenuating effects on excitatory glutamatergic transmission, A1R activation has also been found to potentiate spinal glycinergic neurotransmission and enhance spinal glycinergic inhibition, thus suppressing nociceptive transduction (Bai et al., 2017). These results suggest that the underlying antinociceptive effects of A1R may act through modulating the balance between excitatory synaptic (facilitation) and inhibitory synaptic (disinhibition) mechanisms in nociceptive transmission. The present study A1R was co-expressed with NR1 in the ipsilateral SCDH neurons of MIA-injected rats. We also found that p-NR1 was significantly upregulated by intrathecal injection of DPCPX, an antagonist of the A1R, in MIA-injected rats given laser irradiation, which suggests a synergistic effect between laser treatment and A1R activation. As such, we propose that A1R might contribute to the antinociceptive effects of 10.6-μm laser in MIA-induced OA pain, at least in part through inhibition of p-NR1.

Remarkably, our results showed that the 10.6-μm laser significantly upregulated A1R expression at the spinal level in MIA-induced knee OA pain. We further observed that DPCPX markedly blocked the effects of laser treatment that were demonstrated by PWMT and weight-bearing difference behavioral tests, suggesting that A1R might contribute to the antinociceptive effects of a 10.6-μm laser at the spinal level in MIA-induced OA pain.

The research highlights several outstanding questions for future investigation. We only attempted a preliminary investigation into the role of the A1R in the analgesic effect of 10.6-μm laser treatment, which may partly act by increasing A1R binding and decreasing p-NR1 to alleviate pain. Further research is required to specify the mechanisms by which A1R and p-NR1 interact as a result of 10.6-μm laser therapy. Activation of the spinal A1R results in changes in both excitatory and inhibitory synaptic transmission,

but the current study only focused on the interactions between A1R and NR1 in the MIA-induced knee OA model. Therefore, A1R-related downstream signaling pathways and the interactions of A1R with both glutamatergic and glycinergic neurons in nociceptive transduction at the spinal level are worthy of study. Further research of the synergistic effects of 10.6-μm laser and A1R is a crucial step towards overcoming the limitations of systemically-administered A1R agonists, cardiovascular side effects of which limit their clinical use.

5. Conclusions

The current study provides direct evidence that 10.6-μm laser irradiation at ST35 acts through the upregulation of spinal A1R to inhibit nociception and p-NR1 in the MIA-induced knee OA model. This may be a potential underlying mechanism of 10.6-μm laser analgesia in the management of osteoarthritis pain.

Author contributions

YL performed laser treatment, Western blot, and immunofluorescence analysis. FW conducted behavioral tests. JZW analyzed the data. YL, LX L, and XYS designed the study. YL drafted the manuscript. LXL and XYS revised the manuscript.

Ethics approval and consent to participate

All the experimental procedures conformed to the guidelines for the care and use of the laboratory animal. They were approved by the Animal Welfare and Ethics Committee of the Shanghai University of Traditional Chinese Medicine (PZSHUTCM18113003).

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Conflict of Interest

The authors declare no conflict of interest.

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