Analgesic effects and arthritic changes following tramadol administration in a rat hip osteoarthritis model

（ラット変形性股関節症モデルにおける Tramadol 投与による除痛効果および関節症性変化についての検討）

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Abstract

We investigated the analgesic effects of tramadol and the arthritic changes following tramadol administration in the rat hip osteoarthritis (OA) model using mono- iodoacetate (MIA). The right hip joints of male Sprague–Dawley rats (n = 5 rats/ group) in the Sham group were injected with 25 μl of sterile saline and 1% of fluorogold (FG) retrograde neurotracer. In the MIA + Vehicle and MIA + Tramadol groups, FG and 25 μl of sterile saline with 0.5 mg of MIA were injected into the right hip joint. The MIA + Vehicle and MIA + Tramadol groups were administered daily for 4 weeks, either sterile saline (10 mg/kg, intraperitoneal [i.p.]) or tramadol (10 mg/kg, i.p.). We assessed hyperalgesia every week after MIA administration. Histopathological changes and immunoreactive neurons for calcitonin gene-related peptide (CGRP) in dorsal root ganglia (DRG) were evaluated after 4 weeks of treatment. MIA injection into the hip joint led to mechanical hyperalgesia ($p < 0.01$), which was significantly reduced by tramadol administration ($p < 0.01$). Furthermore, daily i.p injection of tramadol significantly suppressed CGRP expression in DRG ($p < 0.0001$). MIA + Vehicle and MIA + Tramadol groups showed significant cartilage reduction and degeneration compared to the Sham group ($p < 0.0001$). Interestingly, OA changes significantly progressed in the MIA + Tramadol group compared to the MIA + Vehicle group ($p < 0.0001$).

Keywords: hip osteoarthritis, opioid, pain, rat MIA-induced OA model, tramadol

Introduction

Osteoarthritis (OA) is highly prevalent worldwide; it is a leading cause of disability and negatively impact the physical and mental well-being of affected patients [1]. Pain, the main symptom of OA and occurs more often than stiffness or disability [2]. In patients with OA pain, joint pathology does not always reflect the degree of pain. Reportedly, up to 40% of patients with radiographic OA findings have no pain, whereas patients with slight OA indications in radiographic examinations experience severe to debilitating pain [3]. However, the mechanism of OA-associated pain has not yet been fully understood. Various animal models have been reported for knee OA, such as models induced by anterior cruciate ligament transection [4] and partial medial meniscectomy [5]. Mono-iodoacetate (MIA) is frequently used to chemically induce OA in rat models via intra-articular injection to the knee [6, 7]. Since there are only few surgically-induced hip joint models, an animal model using MIA has recently been developed. It has been reported that an intraarticular MIA injection to the hip joint [8] led to the expression of calcitonin gene-related peptide (CGRP), which functions as a mediator of peripheral neurogenic inflammation [9], and activation of transcription factor 3, which is usually regarded as a neuronal damage marker [10-12] expressed late in the dorsal root ganglia (DRG) of the rat hip, thus reflecting the involvement of both neurogenic inflammation and local nerve damage in the rat OA model. It has also been reported that duloxetine exerts a pain-relieving effect on neuropathic pain in this animal model [13].

Tramadol is a weak opioid that acts on μ-opioid receptors, with a lower affinity for the receptor than morphine [14]. Tramadol is widely recognized and used for relieving pain in OA [15, 16]. Animal studied have demonstrated the pain-relieving effect of tramadol in an MIA-induced knee OA model [14, 17]. In contrast, studies on the effect of opioid administration on arthritic changes suggest that OA progresses with the use of strong opioids over a relatively short period in humans [18]. Therefore, there is a hypothesis that excessive pain relief promotes arthritic changes. Although tramadol is clinically used more frequently than potent opioids in OA treatment, to the best of our knowledge, no study has reported whether the excessive use of tramadol for pain relief accelerates OA progression. Therefore, the purpose of this study was to investigate the pain-relieving effects of tramadol and the progression of osteoarthritic changes using a rat hip OA model induced by MIA.

Methods

Ethical Approval

All animal testing protocols were reviewed and approved by the ethics committee of the Chiba University Hospital (Chiba, Japan). All animal experiments adhered to the National Institutes of Health guidelines for the management and use of laboratory animals.

Intra-Articular Injection of MIA and Retrograde Neurotracing

We used 20 6-week-old male Sprague-Dawley rats weighing 250-300 g (CLEA, Tokyo, Japan). The rats were housed in a semi-barrier system with a controlled environment (12 h light/dark cycle,

temperature: 21-23 °C, and humidity: 45-65%). All rats were given water and food *ad libitum* and were fed a standard rodent chow diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). Based on previously published research [19], all rats were anesthetized with an intraperitoneal (i.p.) injection of 0.3 mg/kg of medetomidine (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 4.0 mg/kg of midazolam (Maruishi Pharmaceutical. Co., Ltd., Osaka, Japan), and 5.0 mg/kg of butorphanol (Meiji Seika Pharma. Co., Ltd., Tokyo, Japan) and treated aseptically throughout the experiments. The control (Sham) group comprised 5 rats, which received 25 μL of sterile saline injection into their right hip joints. The experimental group comprised 10 rats, which received 0.5 mg of MIA injection into their right hip joints using a 27-gauge needle [8, 20]. The remaining 5 rats were assigned to the Sham + Tramadol group (n = 5). Sham and the 10 experimental rats received 1% fluorogold (FG) retrograde neurotracer injections into their right hip joints. Two weeks after surgery, the 10 experimental rats were randomly placed into treatment groups that received either saline (MIA + Vehicle group, n = 5: 10 mg/kg, i.p.) or tramadol (MIA + Tramadol group, n = 5: 10 mg/kg, i.p.) daily for 4 weeks. The rats in the Sham group were only administered the vehicle. The rats in the Sham + Tramadol group were administered tramadol (10 mg/kg, i.p.) daily for 4 weeks to evaluate the effects of i.p. administration of tramadol on the hip joints. Tramadol was dissolved in 0.5 mL saline, and its dose was determined based on previous studies [21, 22].

Behavioral Tests

We evaluated mechanical cutaneous plantar sensitivity using the von Frey assay every week for 4 weeks after treatment in a behavioral study. Five rats in each of the three groups (Sham, MIA + Vehicle, and MIA + Tramadol) were subject to behavioral testing. The rats were randomly selected and allowed to adapt to the test chamber for 1 h before performing behavioral tests. Baseline thresholds were acquired before MIA induction. Two weeks after surgery, behavioral testing was performed weekly for 4 weeks. Calibrated von Frey filaments (Monofilament kit; Smith & Nephew, Watford, UK) were applied for 4 s or until withdrawal (whichever occurred first) and the 50% paw withdrawal threshold (PWT, g) was calculated [23]. The stimulus intensity ranged from 1 to 60 g, corresponding to the number of filaments (4.08, 4.17, 4.31, 4.56, 4.74, 4.93, 5.07, 5.18, 5.46, and 5.88). For each animal, the actual filaments used within the previously mentioned series were identified based on the lowest filament to elicit a positive response, followed by five consecutive stimulations using the up-down method [24, 25]. The filament range and average spacing were incorporated into individual threshold calculations, along with the response pattern. Mechanical hypersensitivity on the plantar surface area of the right (ipsilateral) hind paw was assessed using a wire mesh observation cage. Data are presented as the 50% PWT for each group ± standard error of the mean.

Immunohistochemical Expression of CGRP

After the transcardiac perfusion with 0.9% saline, followed by 500 mL of 4% paraformaldehyde in

phosphate buffer (0.1 M, pH 7.4), the right DRG in the three groups (Sham, MIA + Vehicle, and MIA + Tramadol) from the L4 levels were resected 4 weeks after tramadol or vehicle administration. DRG specimens were immersed in phosphate-buffered paraformaldehyde overnight at 4 °C. They were frozen in liquid nitrogen after storage in 0.01 M phosphate-buffered saline (PBS) consisting of 20% sucrose for 20 h at 4 °C. Using a cryostat, the DRG were sliced into 10-µm-thick sections (CM3050S; Leica Microsystems, Wetzlar, Germany). Consequently, sections were mounted on poly-L-lysine-coated slides. The specimens were then treated with a non-specific binding site-blocking solution comprising PBS with 0.3% Triton X-100 and 3% skim milk for 90 min at room temperature. Specimens were processed for CGRP immunohistochemistry using a rabbit antibody against CGRP (1:1,000; Chemicon, Temecula, CA, USA). After incubation with the diluted antibody for 20 h at 4 °C, the DRG sections were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (for CGRP immunoreactivity, 1:1,000; Molecular Probes, Eugenem, OR). After each step, the sections were rinsed three times with PBS. The immunostained sections were observed using a fluorescence microscope (Olympus, Tokyo, Japan) in a treatment-blinded manner. The numbers of FG-labeled CGRP-immunoreactive (ir) DRG neurons were counted by blinded observers, and their proportion relative to the total number of FG-labeled DRG neurons was calculated for each DRG sample.

Histopathological Findings

For histological evaluation, samples from all groups were obtained to assess OA progression after MIA administration (after 4 weeks of tramadol or vehicle administration). The rats were anesthetized intraperitoneally with 0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, and 5.0 mg/kg butorphanol. Next, they were perfused transcardially with 0.9% saline, followed by 500 mL of 4% paraformaldehyde in phosphate buffer fixative (0.1 M, pH 7.4). Soft tissues around the right hip joint, such as the cartilage, synovium, and capsule, were resected. The resected limb was cut in the middle of the femur, and the center of the femoral head was immersed in 10% neutral buffered formalin for 3 days. The specimens were continuously demineralized with the reagent K-CX (FALMA, Tokyo, Japan) for 30 h and 5% sodium sulfate for 16 h, and then embedded in paraffin to prepare coronary sections. The samples were continuously sectioned in 8 μm steps and stained with hematoxylin and eosin, safranin O, and toluidine blue. Osteoarthritic changes were evaluated using the Osteoarthritis Research Society International (OARSI) histopathology scoring system [25]. For each joint, we scored 10 slices centered on the maximum diameter of the femoral head. Each sample was evaluated by the depth of change (grading) and width (staging) of OA, and the score was finally expressed by multiplying the depth and width. The average scores for each group were compared between groups.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, San

Diego, CA, USA). The PWT, OARSI scores, and proportions of CGRP-ir FG-labeled neurons in the DRG among groups were compared using two-way analysis of variance, followed by Tukey's multiple comparison test. Statistical significance was set at $p < 0.05$.

Results

Behavioral Tests

Compared to the Sham group, the behavioral pain tests showed that rats in the MIA + Vehicle group showed significant mechanical hypersensitivity every week for 4 weeks after treatment ($p < 0.01$; Fig. 1). In contrast, the MIA + Tramadol group showed significant improvement compared to the MIA + Vehicle group every week after the i.p. administration of tramadol ($p < 0.01$).

Immunohistochemical Expression of CGRP

After 4 weeks of tramadol or vehicle administration, the MIA + Vehicle and MIA + tramadol groups presented significantly higher expression levels of FG-labeled CGRP-ir DRG neurons in L4 than the Sham group ($p < 0.0001$; Fig. 2). Furthermore, the proportion of FG-labeled CGRP-ir DRG neurons in the MIA + Tramadol group was significantly lower than that in the MIA + Vehicle group ($p < 0.0001$).

Histopathological Findings

The MIA + Vehicle and MIA + Tramadol groups presented progressive OA changes, whereas the

Sham and Sham + Tramadol groups maintained a normal appearance (Fig. 3). The OARSI score was higher in the MIA + Vehicle group than in the Sham group ($p < 0.0001$; Fig. 4). Furthermore, compared to the MIA + Vehicle group, OA changes were significantly more pronounced in the MIA + Tramadol group ($p < 0.0001$; Fig. 4).

Discussion

In this study, mechanical hypersensitivity was observed in the MIA + Vehicle group. The expression of CGRP in the DRG was significantly higher in the MIA + Vehicle group than in the Sham group. Histologically, progressive arthritic changes were observed in the 0.5 mg MIA + Vehicle group than in the Sham group. Furthermore, the MIA + Tramadol group showed lower mechanical hypersensitivity than that in the MIA + Vehicle group, and CGRP expression in DRG was suppressed. Histologically, advanced arthritic changes were observed in the MIA + Tramadol group than the MIA + Vehicle group. While these results established the pain-relieving effect of intraperitoneal tramadol, they were also associated with the progression of OA changes. Tramadol is a centrally acting analgesic with many modes of action. It acts on serotonergic and noradrenergic nociception, while its metabolite O-desmethyltramadol acts on the μ-opioid receptor [26]. Tramadol also acts as a serotonin-norepinephrine reuptake inhibitor (SNRI) [27]. Tramadol exhibits concentration-dependent analgesic effects in pain induced by the tail-flick test and hot plate test that

measure heat nociception latency [28] and in pain measured by the Randall-Sllito-typed pressure nociception threshold [29]. Moreover, the effectiveness of tramadol has also been confirmed in other pain behaviors caused by tissue inflammation, such as an inflammatory pain model induced by complete Freund's adjuvant (CFA), and in neuropathic pain due to peripheral nerve injuries, such as chronic constriction injury (CCI) model and spinal nerve ligation (SNL) model [30, 31]. In the present study, the MIA + Vehicle group had increased mechanical hypersensitivity compared with that in the Sham group, and the MIA + Tramadol group showed improved mechanical hypersensitivity relative to that in the MIA + Vehicle group. These findings indicate that the hip pain induced by administration of 0.5 mg MIA in rats. Previous studies reported that the rat hip OA model induced by 2.0 mg of MIA exhibits peripheral inflammation, local nerve damage, and central sensitization, thus leading to chronic pain [13, 32, 33]. Rachel et al. [14] reported that tramadol administration improved mechanical hypersensitivity in the knee of MIA-induced OA model animals. Ishikawa et al. [17] similarly reported that tramadol administration improved the gait paradigm in a rat knee OA model induced by MIA. Our results showed that tramadol was effective against hyperalgesia in the rat hip OA model induced by 0.5mg MIA-administration, similar to the pain phenotype seen with 2.0 mg MIA-administration in hip OA model in rats.

In the MIA + Vehicle group, the expression of CGRP-ir DRG neurons was significantly higher than that in the Sham and MIA + Tramadol groups. DRG neurons are considered to be

responsible for acute inflammatory pain [33]. CGRP reportedly reflects acute inflammatory pain [34] in the knee of an MIA-induced OA rat model [35, 36]. These findings indicate that local inflammation occurred in the hip of an MIA-induced OA model rat, and CGRP expression in the DRG was suppressed by tramadol administration, which is consistent with our study findings that tramadol administration reduced CGRP expression. In addition, immunohistochemistry of peripheral nerve biopsies harvested from patients with Morton's neuroma, which results in neuropathic pain, showed an increased amount of CGRP in patients compared to controls [37]. Schou et al. [38] performed a meta-analysis of CGRP in terms of its role in chronic pain, which indicated that CGRP is involved in neuropathic and chronic pain in addition to inflammation induced pain. In the current study, tramadol effectively reduced the proportion of CGRP-ir DRG neurons and associated mechanical hypersensitivity significantly in the rat hip OA model. Therefore, it is possible that this study model involves elements of chronic pain and neuropathic pain in addition to inflammatory pain. However, further investigations are necessary to ascertain these inferences.

In this study, the MIA + Vehicle group showed OA associated changes, such as degeneration of articular cartilage and decreased cartilage matrix 6 weeks after MIA injection with a mean OARSI score was 10.0 ± 2.0. Kawarai et al. [13] reported that OA associated changes, such as degeneration and fibrosis, subchondral bone collapse, and decreased cartilage matrix were observed 4 weeks after administration of 2.0 mg of MIA and the mean OARSI score was 22.7 ± 1.9. The rat

hip OA model induced by 0.5 mg MIA injection in this study showed a comparatively mild pathological phenotype. Therefore, we decided to use this mild OA model to compare the degree of OA change. While tramadol suppressed mechanical hypersensitivity and local inflammation in the rat hip OA model, histologically, the MIA + Tramadol group demonstrated OA progression compared to the MIA + Vehicle group. The use of opioids for OA pain has previously been reported. Tapentadol or oxycodone treatment was effective for managing moderate-to-severe chronic OA-related knee and hip pain [39, 40]. In contrast, concerning adverse events, such as constipation, somnolence, nausea, vomiting, dizziness, and itching from opioid use for treating chronic non-cancer pain, including OA associated with opioid use, were reported by Kalso et al. [41]. However, in their study, no progression of joint deformities was reported. Recently, Fujii et al. [18] reported that potent opioids resulted in OA progression in a relatively short time. Their results suggest that although OA patients do not experience pain due to the strong analgesic effects of opioids, joint destruction and progressive OA change, such as Charcot's joints, could occur. Tanezumab, a monoclonal antibody against nerve growth factor (NGF) with pain-relieving effects, has demonstrated efficacy in a pivotal 16-week dose-titration phase 3 study for OA pain [42]. The use of anti-NGF antibodies increased the number of cases requiring joint replacement surgery [42], which may also be due to the excessive pain-relieving effect, resulting in OA progression. Although tramadol is widely used for OA treatment, its involvement in the progression of joint deformities is not completely understood. We

hypothesized that the excessive pain-relieving effect would lead to OA progression. In this study, tramadol promoted OA associated changes in a rat hip OA model, while no effect of tramadol itself causing joint destruction was observed. There are no reports of similar findings in existing animal or human experiments.

Nevertheless, this study has several limitations. In this study, tramadol administration reduced pain in the right limb, increasing weight-bearing on the rat left hip joint, subsequently affecting OA progression. However, it is unclear whether the rat's activity and weight-bearing on the left limb actually increased. In other words, this study does not indicate that the amount of activity increased due to the pain-relieving effect and changes in the progression of OA. However, it was suggested that the pain-relieving effect might be related to OA deformations. Secondly, the follow-up period was short. In this study, histological evaluation was performed 6 weeks after MIA administration (4 weeks after daily tramadol administration). The OA changes progressed in a relatively short time due to the administration of tramadol. However, it is necessary to investigate whether joint deformities progress with the long-term administration of tramadol.

In conclusion, tramadol suppressed mechanical hyperalgesia and CGRP expression at the L4 level in the DRG of rats with MIA-induced hip OA. These results imply that tramadol is effective in the conservative treatment of patients who cannot undergo surgery due to various conditions, such as age and other complications. However, it was shown that while chronic tramadol treatment has a

pain-relieving effect in rats with hip OA, it may also contribute to progressive OA changes. Although tramadol has excellent pain-relieving effects and therefore used in OA treatment, there may be a potential risk of developing joint deformities. Therefore, orthopedic surgeons should consider the possibility of progressive joint deformities when prescribing tramadol to OA patients.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1. Effect of intraperitoneal (i.p.) administration of tramadol on the withdrawal threshold of the pressure applied to the hind paw on the ipsilateral side. Changes in hyperalgesia were determined using von Frey filaments and expressed as the 50% paw withdrawal threshold in grams (g). Behavioral testing was performed before monosodium iodoacetate (MIA) administration (baseline, BL). Then, the animals were injected with 0.5 mg of MIA (MIA + Vehicle and MIA + Tramadol groups) or saline (Sham group) and tested at BL and 1, 2, 3, and 4 weeks after daily i.p. administration of tramadol or vehicle. Lower thresholds indicate increased hyperalgesia. Data are

expressed as the mean ± standard error of the mean for the five rats in each group. *p < 0.01 compared with the MIA + Vehicle group, ŧp < 0.01 the MIA + Tramadol group compared with the MIA + Vehicle group.

Figure 2. Representative fluorescence photomicrographs of the L4 dorsal root ganglia (DRG) neurons after i.p. administration of tramadol or vehicle for 4 weeks. Photomicrographs of (A and B) are from the same section. (A) Fluorogold (FG)-labeled DRG neurons and (B) calcitonin gene-related peptide (CGRP)-immunoreactive (ir) DRG neurons. Arrows indicate FG-labeled CGRP-ir DRG neurons. The proportions of FG-labeled CGRP-ir neurons at the L4 level in the MIA + Vehicle and MIA + Tramadol groups were significantly higher than that in the Sham group (n = 5 rats per group). The proportion of FG-labeled CGRP-ir neurons at the L4 level in the MIA + Vehicle group was significantly higher than that in the MIA + Tramadol group (n = 5 rats per group). ****p < 0.0001.

Figure 3. Histopathology of the hip in the Sham (A–C), Sham + Tramadol (D–F), MIA + Vehicle (G–I), and MIA + Tramadol (J–L) groups after i.p. administration of tramadol or vehicle for 4 weeks. Hematoxylin and eosin (HE; A, D, G, and J), safranin O (SO; B, E, H, and K), and toluidine blue (TB; C, F, I, and L) staining. The MIA + Vehicle and MIA + Tramadol groups showed extensive

areas of cartilage loss, degeneration, and fibrosis, and subchondral bone collapse (G–L), and the MIA + Tramadol group had stronger joint surface detachment and cartilage matrix reduction than the MIA + Vehicle group.

Figure 4. Graphs showing the Osteoarthritis Research Society International (OARSI) score in each group. Osteoarthritic changes were significantly more apparent in the MIA + Vehicle and MIA + Tramadol groups compared to the Sham group. Relative to the MIA + Vehicle group, OA changes were significantly more apparent in the MIA + Tramadol group. *p < 0.0001.

Figure 1

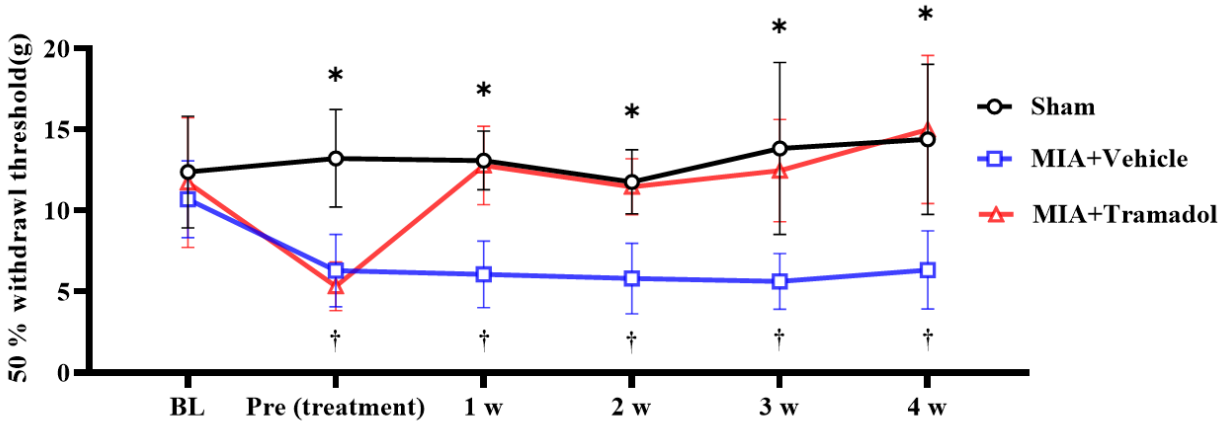


Figure 2

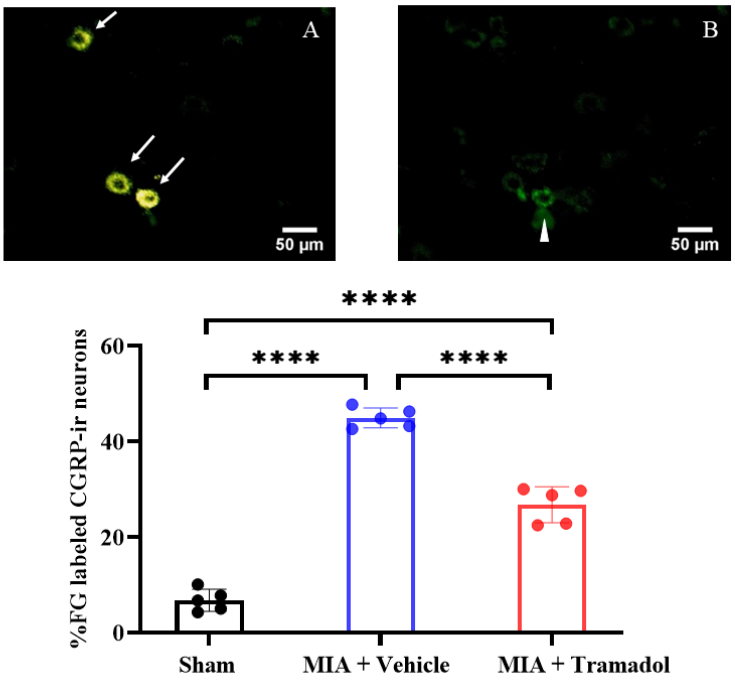


Figure 3

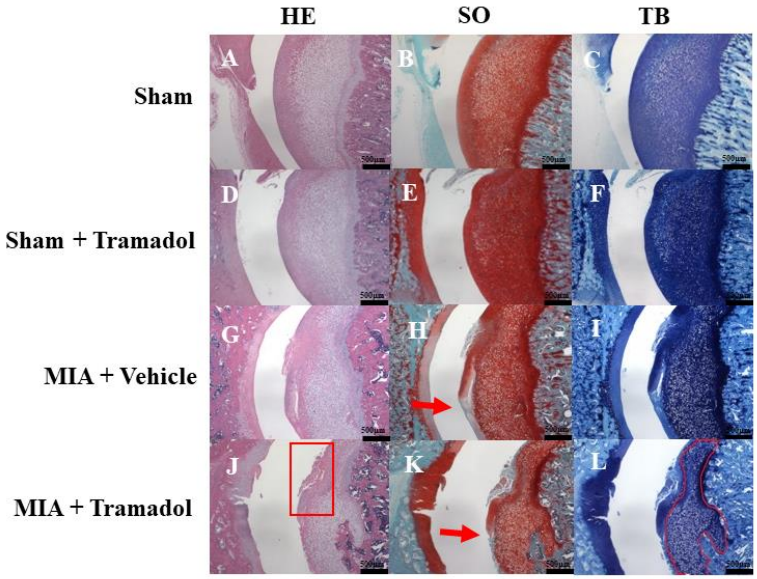
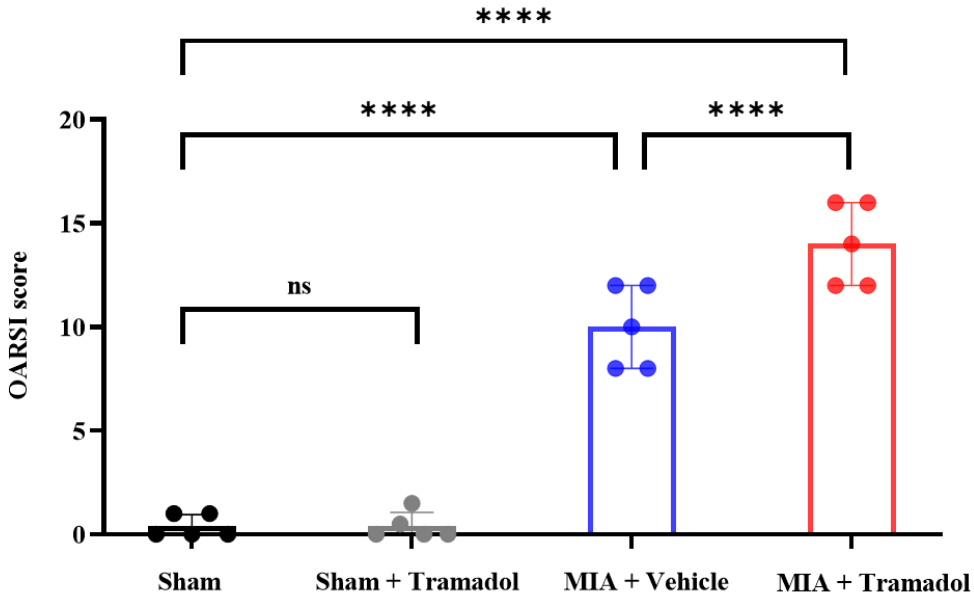


Figure 4


****
****
****
ns
OARSI score
20
15
10
5
0
Sham
Sham + Tramadol
MIA + Vehicle
MIA + Tramadol

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