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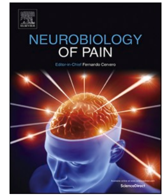

Low-intensity pulsed ultrasound phonophoresis with diclofenac alleviated inflammation and pain via downregulation of M1 macrophages in rats with carrageenan-induced knee joint arthritis

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ABSTRACT

Objective: This study aimed to investigate the effects of low-intensity pulsed ultrasound (LIPUS) phonophoresis with diclofenac on inflammation and pain in the acute phase of carrageenan-induced arthritis in rats.
Design: 60 male Wistar rats were randomly divided into the arthritis, diclofenac, LIPUS, phonophoresis, and sham-arthritis control groups. LIPUS and transdermal diclofenac gel were applied to the lateral side of the inflamed knee for 7 days, initiated postinjection day 1. In the phonophoresis group, diclofenac gel was rubbed onto the skin, followed by LIPUS application over the medication. Knee joint transverse diameters, pressure pain thresholds (PPTs), and paw withdrawal thresholds (PWT) were evaluated. The number of CD68-, CD11c-, and CD206-positive cells, and IL-1β and COX-2 mRNA expression were analyzed 8 days after injection.
Results: In the phonophoresis group, the transverse diameter, PPT, PWT significantly recovered at the day 8 compared to those in the LIPUS and diclofenac groups. The number of CD68- and CD11c-positive cells in the phonophoresis group was significantly lower than that in the LIPUS and diclofenac groups, but no significant differences were observed among three groups in CD206-positive cells. IL-1β and COX-2 mRNA levels were lower in the phonophoresis group than in the arthritis group, although there were no differences among the LIPUS, diclofenac, and phonophoresis groups.
Conclusion: LIPUS phonophoresis with diclofenac is more effective to ameliorate inflammation and pain compared to diclofenac or LIPUS alone, and the mechanism involves the decrease of M1 macrophages.

Introduction

Chronic postsurgical/posttraumatic pain is a global problem that has been reported in 23–30 % of patients with lower extremity fracture(s) and 13–44 % of patients who undergo total knee arthroplasty (Castillo et al., 2006; Sugiyama et al., 2018; Rosenberger and Pogatzki-Zahn, 2022). This phenomenon, which is partially characterized by nociplastic pain (van Driel et al., 2022), is caused by sensitization and plastic changes in the nervous system due to the excessive inflammatory response that occurs after tissue injury (Fitzcharles et al., 2021). In addition, both the severity and duration of inflammation and pain caused by tissue inflammation are risk factors for chronic pain (Fletcher et al., 2015). Therefore, to prevent the development of chronic pain, it is necessary to reduce inflammation and promptly relieve acute pain after tissue injury.

During the inflammatory phase of wound healing, monocytes

Abbreviations: AMPK, adenosine monophosphate-activated kinase; CFA, complete Freund's adjuvant; COX-2, cyclooxygenase-2; IFP, infrapatellar fat pad; IL, interleukin; LIPUS, low-intensity pulsed ultrasound; LIUS, low-intensity ultrasound; NF-κB, nuclear factor kappa B; NSAID, non-steroidal anti-inflammatory drug; PB, phosphate buffer; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PPARγ, peroxisome proliferator-activated receptor gamma; PPT, pressure pain threshold; PWT, paw withdrawal threshold; TNF-α, tumor necrosis factor-α.

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infiltrate the inflamed area and differentiate into M1 macrophages, which produce inflammatory cytokines that induce inflammation and pain (Martinez and Gordon, 2014). In the subacute to late phases of tissue inflammation, the accumulation of macrophages is decreased, and some monocytes and M1 macrophages differentiate into M2 macrophages, which then secrete anti-inflammatory cytokines to decrease inflammation (Xuan et al., 2015). Therefore, the number of M1 macrophages in an inflamed area is a suitable indicator of acute inflammation. In other words, the reduction in M1 macrophages in inflammatory tissues is likely to be a useful intervention strategy for inflammation and pain, and the establishment of a therapeutic plan that targets M1 macrophages is a key factor in preventing the development of chronic pain.

Ultrasound is widely used in medical practice for pain management (Draper et al., 2018; Chen et al., 2022). Low-intensity pulsed ultrasound (LIPUS) does not increase tissue temperature and can be useful during the acute phase of inflammation (de Lucas et al., 2020). Previous studies have demonstrated that LIPUS reduces neutrophil and macrophage infiltration in rat models of complete Freund's adjuvant (CFA)-induced arthritis (Chung et al., 2012, 2016) and cryoinjury (da Silva Junior et al., 2017). In addition, ultrasounds also have the effect of accelerating drug penetration, called phonophoresis (Zhao et al., 2013). Briefly, ultrasound irradiation induces cavitation, which causes subsequent changes in cell membrane permeability (Zhao et al., 2013; Lin et al., 2016; Jiang et al., 2019). In addition, ultrasound vibration generates microstreaming in the affected area and accelerates drug penetration (Zhao et al., 2013). LIPUS phonophoresis has been shown to promote drug penetration into the blood and tissues in previous studies using humans (Rosim et al., 2005) and animals (Herwadkar et al., 2012), which has been shown to relieve inflammation and pain (Yang et al., 2008; Cardoso et al., 2019). Microscopic analysis using hematoxylin and eosin staining showed that LIPUS phonophoresis reduced cellular infiltration, suggesting macrophage accumulation (Cardoso et al., 2019). Meanwhile, interleukin (IL)-1β, an inflammatory cytokine which is produced by macrophages (Bondeson et al., 2006; Takano et al., 2016) and aggravates inflammation and pain (Cunha et al., 1992), is downregulated by both LIPUS (Chung et al., 2012; Feltham et al., 2021) and non-steroidal anti-inflammatory drugs (NSAIDs) (Liu et al., 2018, 2019). Cyclooxygenase (COX)-2 is a prostaglandin synthase produced by macrophages, induced by IL-1β (Iwabuchi et al., 2014), involved in arachidonic acid cascade-induced pain (Cardoso et al., 2019), and a direct target of NSAIDs. COX-2 expression is also downregulated by LIPUS (Nakamura et al., 2010; Chung et al., 2012). In view of these facts, LIPUS phonophoresis in combination with diclofenac and LIPUS may results in a synergistic reduction of leukocyte accumulation and downregulation of both IL-1β and COX-2, leading to a more effective reduction in inflammation and pain. However, these biological effects of LIPUS phonophoresis, especially on the accumulation and differentiation of macrophages and the subsequent expression of IL-1β and COX-2, remain unknown. Hence, we aimed to (1) clarify the effects of LIPUS phonophoresis with transdermal NSAIDs on inflammation and pain and (2) investigate the effects of LIPUS phonophoresis on macrophages and inflammatory mediators.

Materials and methods

Animals

Sixty 8-week-old male Wistar rats (CLEA Japan, Tokyo, Japan) were housed (one or two per cage) at 22 ± 2 °C in a 12-hour light/dark cycle and allowed access to food and water ad libitum. Their weights were 220–250 g. All experiments were approved by the Ethics Review Committee for Animal Experimentation of Nagasaki University (approval no. 1510051250).

Experimental design

To investigate the effects of LIPUS phonophoresis on acute inflammation and pain, 60 rats were randomly divided into the following five groups: arthritis with no therapeutic intervention (arthritis; n = 12), arthritis with transdermal diclofenac administration (diclofenac; n = 12), arthritis with LIPUS (LIPUS; n = 11), arthritis with LIPUS phonophoresis (phonophoresis; n = 13), and sham-arthritis controls (control; n = 12). All treatments were administered a combination of anesthetic agents (5 ml/kg, ip.), which consisted of 0.375 mg/kg medetomidine hydrochloride (Dorbene, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan), 4.0 mg/kg midazolam (Midazolam, Sandoz K.K., Tokyo, Japan), and 5.0 mg/kg butorphanol (Vetorphale, Meiji Seika Pharma Co., Ltd, Tokyo, Japan). Rats in the control and arthritis groups were anesthetized at the same frequency to minimize any differences in the effects of the anesthesia.

Arthritis

As in previous studies (Ishikawa et al., 2019; Nakabayashi et al., 2016; Sasaki et al., 2021), rats in the arthritis, diclofenac, LIPUS, and phonophoresis groups were injected with 300 μl of a mixture of 3 % kaolin (Wako Pure Chemical Industries, Ltd. Osaka, Japan) and 3 % carrageenan (Sigma Chemical Co., St. Louis, MO, USA) into the right knee joint anteriorly under anesthesia. In this arthritis model, the erythrocyte sedimentation rate significantly increased 1 day after injection and returned to baseline 7 days after injection (Sasaki et al., 2021). Therefore, we defined 7 days post-injection as the acute phase of this model. In the control group, 300 μl of saline was injected as a sham treatment.

Drug administration

Referring to previous study (Hsieh, 2006), rats in the diclofenac and phonophoresis groups were transdermally administered 0.4 g of 1 % diclofenac sodium gel (Voltaren gel, Novartis Pharma Co., Ltd, Tokyo, Japan) on the lateral side of the right knee joint under anesthesia. It was applied once a day, every day for 7 days, and beginning 1 day after injection. After administration, to prevent oral ingestion from licking the applied drug, rats wore an Elizabethan collar (Borges et al., 2014; Sekiguchi et al., 2008). As a placebo treatment, 0.4 g of Vaseline was application on rats in the arthritis, LIPUS, and control groups.

Application of LIPUS

In the LIPUS and phonophoresis groups, LIPUS was irradiated using the Osteotron V device (Ito Physiotherapy & Rehabilitation Co., Ltd, Tokyo, Japan), and was continued for 7 days after the injection. The treatment head, with an effective radiating area of 0.8 cm^2, was attached to the lateral side of the right knee joint. LIPUS was applied under the following conditions: stimulation frequency, 1.5 MHz; stimulation intensity, 60 mW/cm^2; duty cycle, 1:4 (non-thermal condition); and application time, 10 min under anesthesia. Commercial ultrasound transmission gel was used as the coupling agent. Additionally, in the phonophoresis group, LIPUS was applied immediately after transdermal diclofenac application.

Behavioral testing

Behavioral testing was performed between 9:00 am and 17:00 pm in a quiet room. During the experimental period, we measured swelling of the right knee joint, pressure pain threshold (PPT) in the right knee joint, and paw withdrawal threshold (PWT) of the right hind paw, which was carried out by two evaluators blinded to the experimental groups.

On all days, we initially evaluated the PPT and PWT during awakening. Then, the rats were anesthetized and their knee joint swelling was measured. Finally, we administered the drug and/or LIPUS. Before measuring PPT and PWT, the rats were allowed to rest for at least 10 min. All rats were allowed to acclimatize to the laboratory environment

for 1 week before study initiation to allow for the evaluations to be done under awakening.

Knee joint swelling

To track changes in joint swelling, we measured the transverse diameter of the inflamed knee joint using Vernier calipers under anesthesia. The transverse diameter was measured in the supine position, and the knee joint was held in its maximum extended position (Ishikawa et al., 2019; Nakabayashi et al., 2016; Sasaki et al., 2021). We measured the distance of the medial–lateral joint fissure and considered this the transverse diameter.

PPT of the right knee joint

The PPT of the right knee joint was assessed using the Randall-Selitto apparatus (Ugo Basile SRL, Lombardia, Italy) equipped with a handmade round-headed probe (tip diameter = 8 mm) (Oga et al., 2020). Rats were lightly restrained by hand. The probe was applied to the lateral side of the right knee joint. The pressure was increased at a constant rate of 48 g/s until the animal withdrew its limb or vocalized. Seven measurements were taken at intervals of at least 30 s, and the mean values excluding the maximum and minimum values were recorded as the PPT.

Mechanical hyperalgesia

Mechanical hyperalgesia of the hind paw was evaluated using the digital von Frey test (IITC-2391; NeuroScience, Tokyo, Japan). Rats were individually placed in a handmade translucent Plexiglas box. Then, the probe was attached below the plantar surface of the hind paw with increasing pressure at a constant rate of 3 g/s, until the animal withdrew its limb or vocalized (Ishikawa et al., 2019, 2021; Nakabayashi et al., 2016; Sasaki et al., 2021). Seven measurements were taken at intervals of at least 30 s, and the mean values excluding the maximum and minimum values were recorded as the PWT.

Tissue sampling and preparation

After the final behavioral testing on day 8, which was approximately 24 h after the final intervention, the rats were deeply anesthetized. Subsequently, the right knee joint was excised from some rats, and the infrapatellar fat pad (IFP) was removed. The knee joints were fixed with 4 % paraformaldehyde dissolved in 0.1 M phosphate buffer (PB; pH 7.4), and then decalcified with 10 % ethylenediaminetetraacetic acid in 0.1 M PB (pH 7.4). The knee joint was dehydrated with a graded series of ethanol solutions and embedded in paraffin. IFP was treated with RNAlater (Ambion, Foster City, CA, USA) immediately after excision and stored in a deep freezer (-80 °C).

Analysis of macrophages in the knee joint synovium

We analyzed the number of macrophages in the knee joint synovium using immunohistological staining. Briefly, longitudinal 5-μm serial sections were cut with a microtome, deparaffinized in xylene, and rehydrated through graded concentrations of alcohol. Sections were then postfixed with 4 % paraformaldehyde dissolved in 0.01 M phosphate buffered saline (PBS) for 15 min and then subjected to an antigen retrieval step by incubation in 0.01 M citrate acid buffer (pH 6.0). Endogenous peroxidase activity was inhibited using 0.6 % H_2O_2 in methanol for 30 min. Sections were blocked with 1 % bovine serum albumin in PBS for 60 min and incubated overnight at 20 °C with mouse monoclonal anti-CD68 primary antibody (1:3000 mouse, BIO-RAD), rabbit polyclonal anti-CD11c antibody (1:2000 rabbit, Funakoshi) and rabbit polyclonal anti-CD206 antibody (1:2000 rabbit, Abcom) as markers for the total, M1, and M2 macrophages, respectively. The sections were rinsed in PBS and incubated with a secondary antibody for 60 min at 20 °C. Each section was stained using an avidin–biotin complex method (Vectastain Elite ABC kit; Vector Laboratories) for 60 min at 20 °C. The sections were then visualized with a 3,3-diaminobendine solution. After the final washing step, each section was stained with 1 % methyl green. Each section was examined under a light microscope, and the entire anterior and posterior capsules were photographed at 400 × magnification using a digital camera. In this study, we investigated the regions within 100 μm of the synovial intima to the outside of the anterior/posterior capsules. We measured the area and counted the number of CD68-positive, CD11c-positive, and CD206 positive cells in the area. Finally, the number of CD-68 positive, CD11c-positive, and CD206-positive cells per 1 mm^2 in the area was calculated. This analysis was performed using ImageJ software. Two sections per rat were used for analysis.

Analysis of IL-1β and COX-2 mRNA in the IFP

We analyzed IL-1β and COX-2 mRNA expression using real-time reverse transcription polymerase chain reaction (PCR). Total RNA was extracted from IFP samples using the RNeasy Fibrous Tissue Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Total RNA was used as a template with a QuantiTect Reverse Transcription Kit (Qiagen) to prepare cDNA, and PCR was performed using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA). The mRNA expression levels were determined using real-time reverse transcription-PCR, which was performed in an optical 96-well plate with an Mx3005P Real-Time QPCR System (Agilent Technologies). The relative expression of IL-1β and COX-2 mRNA was normalized to that of the internal control (β-actin) with ΔΔCt. The following primer pairs were used: IL-1β forward, 5′-AAT-GACCTGTTCTTTGAGGCTGAC-3′ and reverse, 5′-CGA-GATGCTGCTGTGAGATTTGAA-3′. COX-2 forward, 5′-CCCACTTCAAGGGAGTCT-3′ and reverse, 5′- GTGATCTGGACGT-CAACA-3′. β-actin forward, 5′-GTGCTATGTTGCCCTAGACTTCG-3′ and reverse, as well as 5′-GATGCCACAGGATTCCATACCC-3′.

Statistical analysis

All data are presented as mean ± SD. For all data, differences between groups were assessed using a one-way analysis of variance followed by the Bonferroni post hoc test. Values of $P < 0.05$ were considered statistically significant.

Results

Changes in knee joint swelling

One day after injection, the transverse diameter of the right knee joint in the arthritis, diclofenac, LIPUS, and phonophoresis groups significantly increased compared to that in the control group, and there were no significant differences among the four groups (Fig. 1). The knee joint widths at 2 days after injection were significantly decreased in the diclofenac, LIPUS, and phonophoresis groups compared to the arthritis group. Additionally, knee joint widths were significantly decreased in the phonophoresis group compared to the diclofenac and LIPUS groups 7 days after injection, and no significant difference was observed from 7 days after injection onward compared to the control group.

PPTs of the knee joint

In the arthritis, diclofenac, LIPUS, and phonophoresis groups, the PPT of the knee joints 1 day after injection was significantly lower than that of the control group, and there were no significant differences among the four groups (Fig. 2). In the diclofenac, LIPUS, and phonophoresis groups, the PPT was recovered 2 days after injection, and significant differences were observed compared to the arthritis group. Additionally, there were no differences between the changes in the PPT of the diclofenac and LIPUS groups. Furthermore, it was significantly

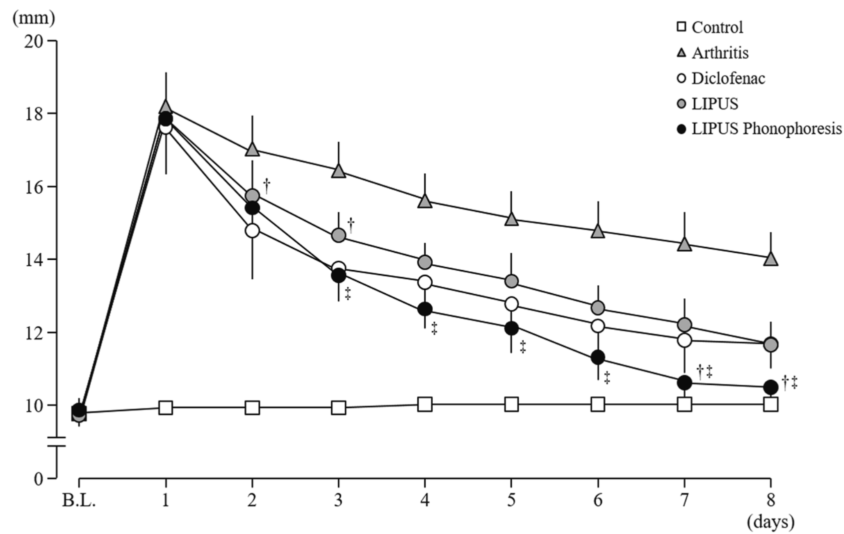

Fig. 1. Time course changes in the transverse diameters of the right knee joints. The data are presented as mean ± standard deviation. † $p < 0.05$ significantly different from the diclofenac group. ‡ $p < 0.05$ significantly different from the LIPUS group.

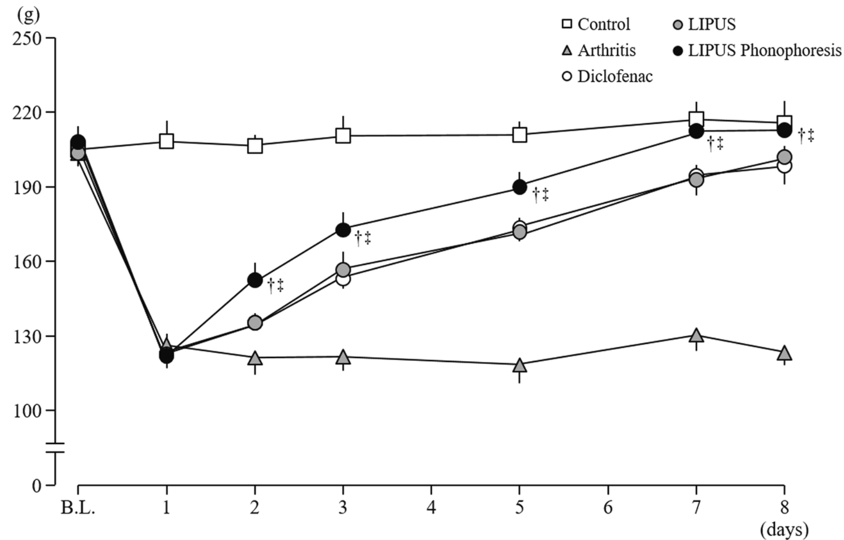

Fig. 2. Time course changes in the PPT of the right knee joints. The data are presented as mean ± standard deviation. † $p < 0.05$ significantly different from the diclofenac group. ‡ $p < 0.05$ significantly different from the LIPUS group.

recovered in the phonophoresis group compared to that in the diclofenac and LIPUS groups 2 days after injection, and no significant difference was observed from 7 days after injection onward compared to the control group.

PWT of the hind paw

The PWT of the Arthritis, diclofenac, LIPUS, and phonophoresis groups was significantly decreased 1 day after injection compared to that of the control group (Fig. 3). In the arthritis group, a significant decrease in PWT was noted throughout the experimental period compared to that in the control group. The PWT in the diclofenac, LIPUS, and phonophoresis groups was significantly higher than that in the arthritis group from 2 days after injection. In addition, there were no significant differences between the diclofenac and LIPUS groups throughout the experimental period. It was significantly increased in the phonophoresis group compared to that in the diclofenac and LIPUS groups 2 days after injection. No significant difference was observed from 7 days after injection onward compared to the control group.

Expression of macrophages in the knee joint synovium

The number of CD68 positive cells per unit area (mm^2) was significantly higher in the arthritis, diclofenac, LIPUS, and phonophoresis groups than in the control group (Fig. 4a). In the diclofenac, LIPUS, and phonophoresis groups, the number of CD68 positive cells was significantly lower than that in the arthritis group. Additionally, no significant difference was observed between the diclofenac and LIPUS groups. Furthermore, the number of CD68 positive cells in the phonophoresis group was significantly lower than that in the diclofenac and LIPUS group.

The number of CD11c positive cells per unit area was almost same results with CD68 positive cells (Fig. 4b). Briefly, in the diclofenac, LIPUS, and phonophoresis groups, the number of CD11c positive cells was significantly lower than that in the arthritis group. Additionally, no significant difference was observed between the diclofenac and LIPUS groups. Furthermore, the number of CD11c positive cells in the phonophoresis group was significantly lower than that in the diclofenac and LIPUS group.

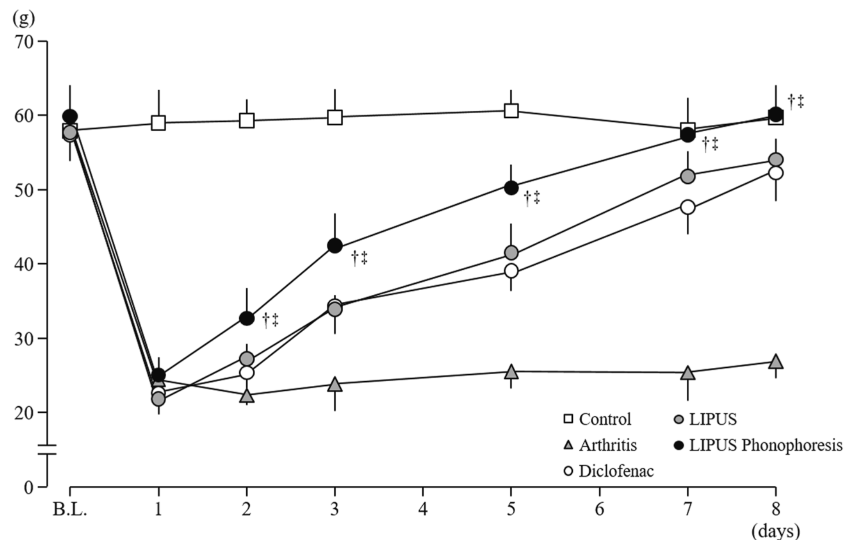

Fig. 3. Time course changes in the PWT of the right hind paws. The data are presented as mean ± standard deviation. † $p < 0.05$ significantly different from the diclofenac group. ‡ $p < 0.05$ significantly different from the LIPUS group.

The number of CD206 positive cells per unit area (mm^2) was significantly higher in the arthritis, diclofenac, LIPUS, and phonophoresis groups than in the control group (Fig. 4c). Although the number of CD11c positive cells in the diclofenac and phonophoresis groups were significantly higher than that in the arthritis group, no significant difference was observed among the diclofenac, LIPUS and phonophoresis groups.

IL-1β and COX-2 mRNA expression in the IFP

The expression of IL-1β and COX-2 mRNA showed a similar trend. Briefly, in the arthritis group, the expression of IL-1β and COX-2 mRNA were significantly upregulated compared with that in the control group (Fig. 5a, b). On the other hand, the expression IL-1β and COX-2 mRNA were significantly downregulated in the diclofenac, LIPUS, and phonophoresis groups, compared to arthritis group. In addition, no significant difference was observed among the diclofenac, LIPUS, and phonophoresis groups in the expression of IL-1β and COX-2 mRNA expression.

Discussion

In this study, we investigated the effects of LIPUS phonophoresis with diclofenac on pain reduction immediately after the onset of arthritis in a rat model of knee arthritis, from behavioral, histological, and molecular biological perspectives. Behavioral assessment showed a significant increase in PPT in the LIPUS phonophoresis group compared to the diclofenac or LIPUS alone groups, with no significant difference from the control group 7 days after injection. These results suggest that LIPUS phonophoresis with diclofenac is more useful than either intervention alone for improving inflammation and pain after arthritis. In addition, a significant increase was observed in the PWT of the hind paws in the LIPUS phonophoresis group compared to that in the diclofenac and LIPUS groups, which was similar to the trend observed for PPT. Continuous noxious stimuli caused by strong and long-lasting inflammation can lead to central sensitization in the spinal dorsal horn (Dieppe and Lohmander, 2005; Woolf, 2011), which evokes secondary hyperalgesia in distant areas (Ishikawa et al., 2019; Nakabayashi et al., 2016; Sasaki et al., 2021). In this study, the PWT in the arthritis group at day 7 significantly decreased, suggesting that sensitization occurred in the spinal dorsal horn; however, no significant differences were observed between the control and LIPUS phonophoresis groups. These results suggest that LIPUS phonophoresis may suppress central sensitization in the spinal cord following early alleviation of inflammation in the synovium and subsequent reduction in the input of noxious stimulation to the spinal cord.

Histological analysis is considered the ultimate indicator of synovitis (Krenn et al., 2017), with macrophages being the chosen markers (Gilbert et al., 2018; Hamilton and Tak, 2009; Mulherin et al., 1996; Tak and Bresnihan, 2000). In the present study, we observed a decrease in the number of M1 macrophages. Previous studies have also reported a correlation between the number of macrophages in the synovium and severity of synovitis (Hamilton and Tak, 2009; Mulherin et al., 1996; Tak and Bresnihan, 2000). It has also been reported that the number of M1 macrophages increases under inflammatory conditions (Martinez and Gordon, 2014; da Silva Junior et al., 2017). Based on these reports, our results suggest that diclofenac, LIPUS, and LIPUS phonophoresis can ameliorate synovitis. Moreover, the number of total and M1 macrophages in the LIPUS phonophoresis group was lower than that in the diclofenac and LIPUS groups, which showed a similar trend to that of PPT. It has been suggested that pain relief by LIPUS phonophoresis may be related to a decrease in the number of M1 macrophages. Previous research has shown that diclofenac or LIPUS can downregulate chemokines associated with macrophage accumulation (Kaur and Sanyal, 2011; Chung et al., 2012; Fattahi and Mirshafiey, 2012; Ulubay et al., 2018), as well as lipopolysaccharide and tumor necrosis factor (TNF)-α involved in M1 macrophage differentiation (Bombardo et al., 2018; Liu et al., 2023; Ju et al., 2023). Additionally, it has been shown that LIPUS phonophoresis delivers approximately 2-fold more diclofenac at the plasma level (Rosim et al., 2005) and 10-fold more diclofenac at the synovial level (Cagnie et al., 2003). Thus, LIPUS phonophoresis may have been more effective than either single intervention because of the increased penetration of the drug into inflamed tissues in addition to the anti-inflammatory effects of diclofenac and LIPUS, which may inhibit macrophage accumulation and decrease differentiation into M1 macrophages.

The synovium and IFP are considered constitutive elements of a single anatomofunctional unit, and the expression levels of inflammatory cytokines in the IFP and synovium are equivalent (Belluzzi et al., 2019 a,b). IL-1β is known to aggravate inflammatory symptoms (Bondeson et al., 2006; Hamilton and Tak, 2009; Kapoor et al., 2011; McInnes and Schett, 2007), especially pain (Cunha et al., 1992), which is produced by macrophages (Bondeson et al., 2006; Takano et al., 2016). COX-2 is a prostaglandin synthase produced by macrophages, induced by IL-1β (Iwabuchi et al., 2014), involved in arachidonic acid cascade-

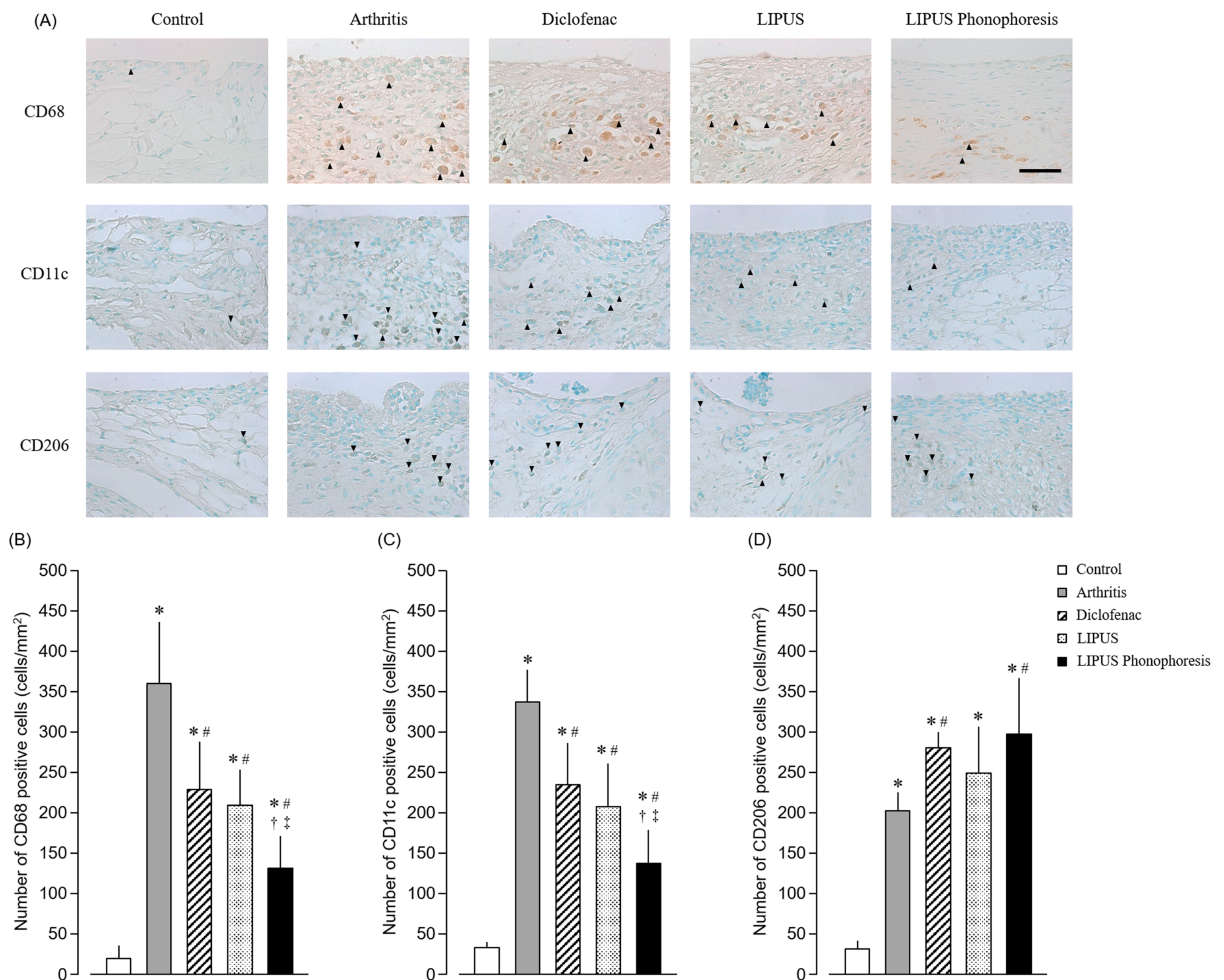

Fig. 4. Immunohistochemical staining for macrophages in the knee joint synovium. Representative photomicrographs of CD68, CD11c, CD206 immunohistochemistry in the knee joint synovium are shown (A). Arrowheads indicate each positive cell. The number of CD68 (B), CD11c (C), CD206 (D) positive cells /mm^2 in the knee joint synovium was calculated. The data are presented as mean ± standard deviation. * $p < 0.05$ significantly different from the control group. # $p < 0.05$ significantly different from the arthritis group. † $p < 0.05$ significantly different from the diclofenac group. ‡ $p < 0.05$ significantly different from the LIPUS group. Scale bar = 50 μm.

induced pain (Cardoso et al., 2019), and a direct target of NSAIDs. In a previous study, LIPUS downregulated IL-1β-induced COX-2 expression (Nakamura et al., 2010), through integrin β1 receptor (Iwabuchi et al., 2014), which acts as a mechanoreceptor on the cell membrane (Takeuchi et al., 2008). Based on previous studies, it was anticipated that LIPUS phonophoresis would concomitantly reduce pain, since both diclofenac and LIPUS have been proven effective in decreasing IL-1β and COX-2, respectively. Our results of real-time RT-PCR, however, suggest that the effects of LIPUS phonophoresis on the expression of IL-1β and COX-2 mRNA were comparable to those of the other two sole interventions, and these changes were not consistent with changes of M1 macrophages. This means that decreases in IL-1β and COX-2 mRNA are associated with the mechanisms involved in reduction of inflammation and pain using LIPUS phonophoresis, but there is no synergistic effect from this strategy, that is, combined therapy using diclofenac and LIPUS. Chung JI, et al. reported that there was a significant correlation between the number of macrophages or expression of IL-1β/COX-2 and inflammatory symptoms, but no correlation between the number of macrophages and IL-1β and COX-2 (Chung et al., 2012). The effects of LIPUS phonophoresis extend beyond the IL-1β and COX-2-mediated pathways and may have synergistically inhibited other pathways. However, we did not examine this, which is an issue for future studies.

The number of M2 macrophages in the diclofenac and LIPUS phonophoresis groups was higher than that in the arthritis group. However, there were no significant differences between the diclofenac and LIPUS phonophoresis groups in this study. Previous studies demonstrated that diclofenac activates the adenosine monophosphate-activated kinase (AMPK) pathway (King et al., 2015), resulting in an increase in the number of M2 macrophages (Park et al., 2017). We speculate that these effects of diclofenac may be related to an increase in the number of M2 macrophages in the diclofenac group. On the other hand, we hypothesized that LIPUS phonophoresis would enhance the anti-inflammatory effect of diclofenac, that is, it would promote an increase in the number of M2 macrophages as well as induce an effect that would promote a decrease in the number of M1 macrophages, but such results were not obtained. LIPUS has been shown to increase the number of M2 macrophages in the early stages of inflammation following muscle injury but not in the later stages (da Silva Junior Em et al., 2017). Thus, the effect of LIPUS alone on the increase in the number of M2 macrophages may be limited to the early stages of inflammation with marked cellular accumulation, which may be related to the lack of a significant increase in M2 macrophage numbers in the LIPUS group in the present study and

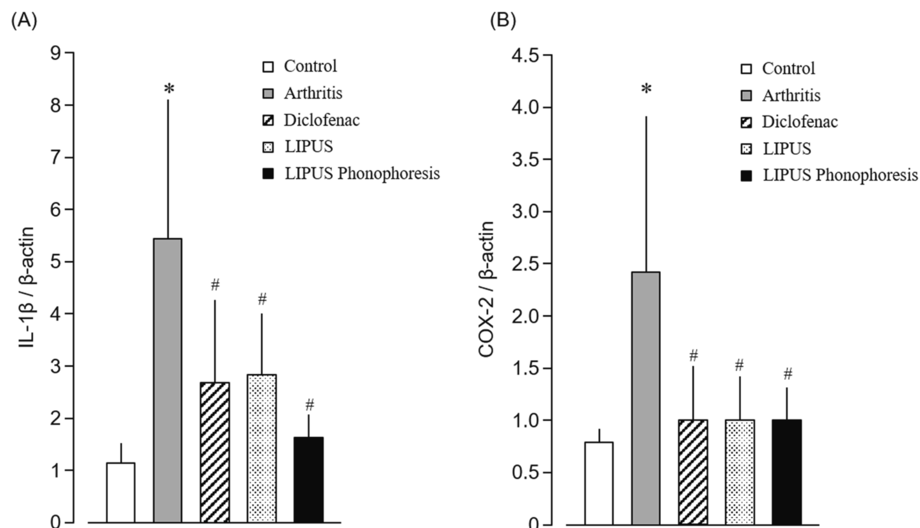

Fig. 5. IL-1β and COX-2 mRNA expression in the infrapatellar fat pad. The data are presented as mean ± standard deviation. * $p < 0.05$ significantly different from the control group. # $p < 0.05$ significantly different from the arthritis group.

the lack of synergism with LIPUS phonophoresis. However, the number of M2 macrophages in the LIPUS phonophoresis group was much greater than that of M1 macrophages, which was more apparent than that in the diclofenac alone and LIPUS alone groups. Thus, LIPUS phonophoresis may be more effective than a single intervention for reducing inflammation. Nevertheless, a detailed study, including a time course, is needed in the future to confirm this hypothesis.

Refractory chronic pain is difficult to manage, and its secondary prevention is recommended (Meyer et al., 2018). Exercise therapy can be beneficial for preventing chronic pain; moreover, it can reduce inflammation and pain and prevent chronic pain in animal models of acute arthritis (Ishikawa et al., 2019). Exercise therapy also activates peroxisome proliferator-activated receptor gamma (PPARγ), which increases M2 macrophage number via IL-4 upregulation (Ruffino et al., 2016; Fernandes et al., 2020; Lu et al., 2021). IL-4 in turn is also involved in increasing the levels of PPARγ, which inhibits the transcriptional activity of nuclear factor kappa B (NF-κB) (Liu et al., 2017), and subsequently reduces M1 macrophage number and IL-1β, IL-6 and TNF-α production (Kawanishi et al., 2022; Murugathasan et al., 2023). IL-10 produced by M2 macrophages further promotes the M2 phenotype and deactivates M1 macrophages (Tidball et al., 2014). Moreover, exercise therapy can reduce calcitonin gene-related peptide levels in the dorsal horn of the spinal cord, indicating suppression of central sensitization (Ishikawa et al., 2019). Therefore, exercise therapy may prevent chronic pain by increasing the number of M2 macrophages and subsequently decreasing the number of M1 macrophages to reduce inflammation at an early stage and suppress central sensitization. However, this study revealed that LIPUS phonophoresis reduced M1 macrophage number, but did not increase M2 macrophage number. Thus, the mechanisms underlying the anti-inflammatory and analgesic effects of exercise therapy and LIPUS phonophoresis are different—the main targets of exercise therapy in suppressing inflammation are M2 macrophages, whereas LIPUS phonophoresis targets M1 macrophages. Patients with severe inflammation after tissue damage often find it difficult to exercise actively, which makes it difficult to derive the corresponding benefits of exercise therapy, such as anti-inflammatory and analgesic effects. Conversely, diclofenac application is not a nociceptive stimulus, and LIPUS generates energy through repeated low-intensity micro vibrations and is not perceived as a stimulus either. Therefore, LIPUS phonophoresis is unlikely to cause treatment-related pain and may reduce inflammation and acute pain by reducing M1 macrophage accumulation from the early stages of inflammation. This would also enable the early application of exercise therapy, which may induce biological responses promoting anti-inflammatory and wound healing, such as an increase in M2 macrophage number, to result in the early reduction of acute pain and prevention of chronic pain. Thus, the combination of LIPUS phonophoresis and exercise therapy may be more effective in preventing chronic pain than either approach in isolation. However, the effects and mechanisms of action of combination LIPUS phonophoresis and exercise therapy require further examination in the future.

This study has some limitations. First, we only investigated IL-1β, but not other inflammatory cytokines. In addition, we investigated IL-1β at the mRNA and not protein level. Further studies are required to clarify the biological effects of LIPUS phonophoresis with diclofenac on inflammation. Second, the analysis of macrophages, IL-1β, and COX-2 was performed only with the endpoints of examination taken into consideration. Third, we did not investigate the factors involved in the central sensitization of the spinal cord, such as the activation of glial cells and the expression of other neurotransmitters, such as substance P, nitric oxide, and glutamate. Finally, the in vivo aspect of this study was based on animals, thus additional clinical research is required to translate these findings into clinical practice.

Conclusions

In summary, LIPUS phonophoresis with transdermal diclofenac onset in the acute phase of arthritis ameliorates inflammation and pain by reducing M1 macrophages. The reduction of pain due to LIPUS phonophoresis partly affected by IL-1β and COX-2, and another pathway might be affected. We believe that LIPUS phonophoresis with transdermal diclofenac is effective in reducing inflammation and acute pain, and may prevent conversion to chronic pain.

CRediT authorship contribution statement

Ryo Sasaki: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft. **Junya Sakamoto:** Conceptualization, Data curation, Funding acquisition, Resources, Validation, Writing – review & editing. **Yuichiro Honda:** Investigation, Methodology. **Satoko Motokawa:** Investigation. **Hideki Kataoka:** Conceptualization, Formal analysis, Validation. **Tomoki Origuchi:** Conceptualization, Resources. **Minoru Okita:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Belluzzi, E., Stocco, E., Pozzuoli, A., Granzotto, M., Porzionato, A., Vettor, R., De Caro, R., Ruggieri, P., Ramonda, R., Rossato, M., Favero, M., Macchi, V., 2019. Contribution of Infrapatellar Fat Pad and Synovial Membrane to Knee Osteoarthritis Pain. Biomed Res Int. 2019, 1–18.

Belluzzi, E., Olivotto, E., Toso, G., Cigolotti, A., Pozzuoli, A., Biz, C., Trisolino, G., Ruggieri, P., Grigolo, B., Ramonda, R., Favero, M., 2019 Mar. Conditioned media from human osteoarthritic synovium induces inflammation in a synoviocyte cell line. Connect Tissue Res. 60 (2), 136–145. https://doi.org/10.1080/03008207.2018.1470167.

Bombardo, M., Malagola, E., Chen, R., Rudnicka, A., Graf, R., Sonda, S., 2018 Jan. Ibuprofen and diclofenac treatments reduce proliferation of pancreatic acinar cells upon inflammatory injury and mitogenic stimulation. Br J Pharmacol. 175 (2), 335–347. https://doi.org/10.1111/bph.13867.

Bondeson, J., Wainwright, S.D., Lauder, S., Amos, N., Hughes, C.E., 2006. The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. Arthritis Res Ther. 8 (6), R187. https://doi.org/10.1186/ar2099.

Borges, G., Neto, F., Mico, J.A., Berrocoso, E., 2014 Jun. Reversal of monoarthritis-induced affective disorders by diclofenac in rats. Anesthesiology. 120 (6), 1476–1490. https://doi.org/10.1097/ALN.0000000000000177.

Cagnie, B., Vinck, E., Rimbaut, S., Vanderstraeten, G., 2003 Aug. Phonophoresis versus topical application of ketoprofen: comparison between tissue and plasma levels. Phys Ther. 83 (8), 707–712.

Cardoso, L.C.P., Pinto, N.B., Nobre, M.E.P., Silva, M.R., Pires, G.M., Lopes, M.J.P., Viana, G.S.B., Rodrigues, L.M.R., 2019 Jan 24. Anti-inflammatory and antinociceptive effects of phonophoresis in animal models: a randomized experimental study. Braz J Med Biol Res. 52 (2), e7773.

Castillo, R.C., MacKenzie, E.J., Wegener, S.T., Bosse, M.J., 2006. Prevalence of chronic pain seven years following limb threatening lower extremity trauma. Pain. 124 (3), 321–329.

Chen, H., Wang, Z., Zhang, X., Sun, M., 2022 Sep. Effects of low-intensity pulsed ultrasound on knee osteoarthritis: A systematic review and meta-analysis of randomized controlled trials. Clin Rehabil. 36 (9), 1153–1169. https://doi.org/10.1177/02692155221097035.

Chung, J.I., Barua, S., Choi, B.H., Min, B.H., Han, H.C., Baik, E.J., 2012 Apr. Anti-inflammatory effect of low intensity ultrasound (LIUS) on complete Freund's adjuvant-induced arthritis synovium. Osteoarthritis Cartilage. 20 (4), 314–322. https://doi.org/10.1016/j.joca.2012.01.005.

Chung, J.I., Min, B.H., Baik, E.J., 2016. Effect of Continuous-Wave Low-Intensity Ultrasound in Inflammatory Resolution of Arthritis-Associated Synovitis. Phys Ther. 96, 808–817.

Cunha, F.Q., Poole, S., Lorenzetti, B.B., Ferreira, S.H., 1992 Nov. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Br J Pharmacol. 107 (3), 660–664. https://doi.org/10.1111/j.1476-5381.1992.tb14503.x.

da Silva Junior, E.M., Mesquita-Ferrari, R.A., França, C.M., Andreo, L., Bussadori, S.K., Fernandes, K.P.S., 2017 Dec. Modulating effect of low intensity pulsed ultrasound on the phenotype of inflammatory cells. Biomed Pharmacother. 96, 1147–1153. https://doi.org/10.1016/j.biopha.2017.11.108.

de Lucas, B., Pérez, L.M., Bernal, A., Gálvez, B.G., 2020 Sep 17. Ultrasound Therapy: Experiences and Perspectives for Regenerative Medicine. Genes (basel). 11 (9), 1086. https://doi.org/10.3390/genes11091086.

Dieppe, P.A., Lohmander, L.S., 2005. Pathogenesis and management of pain in osteoarthritis. Lancet. 365 (9463), 965–973.

Draper, D.O., Klyve, D., Ortiz, R., Best, T.M., 2018 Oct 16. Effect of low-intensity long-duration ultrasound on the symptomatic relief of knee osteoarthritis: a randomized, placebo-controlled double-blind study. J Orthop Surg Res. 13 (1), 257. https://doi.org/10.1186/s13018-018-0965-0.

Fattahi, M.J., Mirshafiey, A., 2012. Prostaglandins and rheumatoid arthritis. Arthritis. 2012, 1–7.

Feltham, T., Paudel, S., Lobao, M., Schon, L., Zhang, Z., 2021 Apr. Low-Intensity Pulsed Ultrasound Suppresses Synovial Macrophage Infiltration and Inflammation in Injured Knees in Rats. Ultrasound Med Biol. 47 (4), 1045–1053. https://doi.org/10.1016/j.ultrasmedbio.2020.12.019.

Fernandes, T.L., Gomoll, A.H., Lattermann, C., Hernandez, A.J., Bueno, D.F., Amano, M. T., 2020 Feb. Macrophage: A Potential Target on Cartilage Regeneration. Front Immunol. 11 (11), 111. https://doi.org/10.3389/fimmu.2020.00111.

Fitzcharles, M.A., Cohen, S.P., Clauw, D.J., Littlejohn, G., Usui, C., Häuser, W., 2021 May 29. Nociplastic pain: towards an understanding of prevalent pain conditions. Lancet. 397 (10289), 2098–2110. https://doi.org/10.1016/S0140-6736(21)00392-5.

Fletcher, D., Stamer, U.M., Pogatzki-Zahn, E., Zaslansky, R., Tanase, N.V., Perruchoud, C., Kranke, P., Komann, M., Lehman, T., Meissner, W., 2015 Oct. euCPSP group for the Clinical Trial Network group of the European Society of Anaesthesiology. Chronic postsurgical pain in Europe: An observational study. Eur J Anaesthesiol. 32 (10), 725–734. https://doi.org/10.1097/EJA.0000000000000319.

Gilbert, S.J., Bonnet, C.S., Stadnik, P., Duance, V.C., Mason, D.J., Blain, E.J., 2018 Feb 17. Inflammatory and degenerative phases resulting from anterior cruciate rupture in a non-invasive murine model of post-traumatic osteoarthritis. J Orthop Res. 36 (8), 2118–2127. https://doi.org/10.1002/jor.23872.

Hamilton JA, Tak PP. The dynamics of macrophage lineage populations in inflammatory and autoimmune diseases. Arthritis Rheum. 2009 May;60(5):1210-21. doi: 10.1002/art.24505.

Herwadkar, A., Sachdeva, V., Taylor, L.F., Silver, H., Banga, A.K., 2012 Feb 28. Low frequency sonophoresis mediated transdermal and intradermal delivery of ketoprofen. Int J Pharm. 423 (2), 289–296. https://doi.org/10.1016/j.ijpharm.2011.11.041.

Hsieh, Y.L., 2006 Jan. Effects of ultrasound and diclofenac phonophoresis on inflammatory pain relief: suppression of inducible nitric oxide synthase in arthritic rats. Phys Ther. 86 (1), 39–49. https://doi.org/10.1093/ptj/86.1.39.

Ishikawa, K., Kajiwara, Y., Sakamoto, J., Sasaki, R., Goto, K., Honda, Y., Kataoka, H., Okita, M., 2019 Jul. Low-intensity muscle contraction exercise following the onset of arthritis improves hyperalgesia via reduction of joint inflammation and central sensitization in the spinal cord in a rat model. Neurosci Lett. 27 (706), 18–23. https://doi.org/10.1016/j.neulet.2019.04.031.

Ishikawa, K., Oga, S., Goto, K., Sakamoto, J., Sasaki, R., Honda, Y., Kataoka, H., Okita, M., Gazerani, P., 2021. Voluntary Forelimbs Exercise Reduces Immobilization-Induced Mechanical Hyperalgesia in the Rat Hind Paw. Pain Res Manag. 2021, 1–8.

Iwabuchi, Y., Tanimoto, K., Tanne, Y., Inubushi, T., Kamiya, T., Kunimatsu, R., Hirose, N., Mitsuyoshi, T., Su, S., Tanaka, E., Tanne, K., 2014. Effects of Low-Intensity Pulsed Ultrasound on the Expression of Cyclooxygenase-2 in Mandibular Condylar Chondrocytes. J Oral Facial Pain Headache 28 (3), 261–268.

Jiang, X., Savchenko, O., Li, Y., Qi, S., Yang, T., Zhang, W., Chen, J., 2019 Oct. A Review of Low-Intensity Pulsed Ultrasound for Therapeutic Applications. IEEE Trans Biomed Eng. 66 (10), 2704–2718. https://doi.org/10.1109/TBME.2018.2889669.

Ju, Z., Shang, Z., Mahmud, T., Fang, J., Liu, Y., Pan, Q., Lin, X., Chen, F., 2023 Apr 28. Synthesis and Anti-Inflammatory Activity of the Natural Cyclooxygenase-2 Inhibitor Axinelline A and Its Analogues. J Nat Prod. 86 (4), 958–965. https://doi.org/10.1021/acs.jnatprod.2c01153.

Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J.P., Fahmi, H., 2011 Jan. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol. 7 (1), 33–42. https://doi.org/10.1038/nrrheum.2010.196.

Kaur, J., Sanyal, S.N., 2011 Sep. Diclofenac, a selective COX-2 inhibitor, inhibits DMH-induced colon tumorigenesis through suppression of MCP-1, MIP-1α and VEGF. Mol Carcinog. 50 (9), 707–718. https://doi.org/10.1002/mc.20736.

Kawanishi, M., Kami, K., Nishimura, Y., Minami, K., Senba, E., Umemoto, Y., Kinoshita, T., Tajima, F., 2022 Oct. Exercise-induced increase in M2 macrophages accelerates wound healing in young mice. Physiol Rep. 10 (19), e15447.

King, T.S., Russe, O.Q., Möser, C.V., Ferreirós, N., Kynast, K.L., Knothe, C., Olbrich, K., Geisslinger, G., Niederberger, E., 2015 Sep. AMP-activated protein kinase is activated by non-steroidal anti-inflammatory drugs. Eur J Pharmacol. 5 (762), 299–305. https://doi.org/10.1016/j.ejphar.2015.06.001.

Krenn, V., Perino, G., Rüther, W., Krenn, V.T., Huber, M., Hügle, T., Najm, A., Müller, S., Boettner, F., Pessler, F., Waldstein, W., Kriegsmann, J., Casadonte, R., Häupl, T., Wienert, S., Krukemeyer, M.G., Sesselmann, S., Sunitsch, S., Tikhilov, R., Morawietz, L., 2017 Aug. 15 years of the histopathological synovitis score, further development and review: A diagnostic score for rheumatology and orthopaedics. Pathol Res Pract. 213 (8), 874–881. https://doi.org/10.1016/j.prp.2017.05.005.

Lin, G., Reed-Maldonado, A.B., Lin, M., Xin, Z., Lue, T.F., 2016. Effects and mechanisms of low-intensity pulsed ultrasound for chronic prostatitis and chronic pelvic pain syndrome. Int J Mol Sci. 17. https://doi.org/10.3390/ijms17071057.

Liu, Y., Duan, C., Chen, H., Wang, C., Liu, X., Qiu, M., Tang, H., Zhang, F., Zhou, X., Yang, J., 2018 Jul. Inhibition of COX-2/mPGES-1 and 5-LOX in macrophages by leonurine ameliorates monosodium urate crystal-induced inflammation. Toxicol Appl Pharmacol. 15 (351), 1–11. https://doi.org/10.1016/j.taap.2018.05.010.

Liu, Y., Tang, H., Liu, X., Chen, H., Feng, N., Zhang, J., Wang, C., Qiu, M., Yang, J., Zhou, X., 2019. Jan. Frontline Science: Reprogramming COX-2, 5-LOX, and CYP4A-mediated arachidonic acid metabolism in macrophages by salidroside alleviates gouty arthritis. J Leukoc Biol 105 (1), 11–24. https://doi.org/10.1002/JLB.3HI0518-193R.

Liu, Z., Li, J., Bian, Y., Zhang, X., Cai, X., Zheng, Y., 2023 May. Low-intensity pulsed ultrasound reduces lymphedema by regulating macrophage polarization and enhancing microcirculation. Front Bioeng Biotechnol. 5 (11), 1173169. https://doi.org/10.3389/fbioe.2023.1173169.

Liu, T., Zhang, L., Joo, D., Sun, S.C., 2017. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2, 17023. https://doi.org/10.1038/sigtrans.2017.23.

Lu, J., Guan, H., Wu, D., Hu, Z., Zhang, H., Jiang, H., Yu, J., Zeng, K., Li, H., Zhang, H., Pan, C., Cai, D., Yu, X., 2021 Jul. Pseudolaric acid B ameliorates synovial inflammation and vessel formation by stabilizing PPARγ to inhibit NF-κB signalling

pathway. J Cell Mol Med. 25 (14), 6664–6678. https://doi.org/10.1111/jcmm.16670.

Martinez, F.O., Gordon, S., 2014 Mar. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 3 (6), 13. https://doi.org/10.12703/P6-13.

McInnes, I.B., Schett, G., 2007 Jun. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 7 (6), 429–442. https://doi.org/10.1038/nri2094.

Meyer, C., Denis, C.M., Berquin, A.D., 2018 Sep. Secondary prevention of chronic musculoskeletal pain: A systematic review of clinical trials. Ann Phys Rehabil Med. 61 (5), 323–338. https://doi.org/10.1016/j.rehab.2018.03.002.

Mulherin, D., Fitzgerald, O., Bresnihan, B., 1996 Jan. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum. 39 (1), 115–124. https://doi.org/10.1002/art.1780390116.

Murugathasan, M., Jafari, A., Amandeep, A., Hassan, S.A., Chihata, M., Abdul-Sater, A. A., 2023 Aug 1. Moderate exercise induces trained immunity in macrophages. Am J Physiol Cell Physiol. 325 (2), C429–C442. https://doi.org/10.1152/ajpcell.00130.2023.

Nakabayashi, K., Sakamoto, J., Kataoka, H., Kondo, Y., Hamaue, Y., Honda, Y., Nakano, J., Okita, M., 2016 Nov 8. Effect of continuous passive motion initiated after the onset of arthritis on inflammation and secondary hyperalgesia in rats. Physiol Res. 65 (4), 683–691. https://doi.org/10.33549/physiolres.933214.

Nakamura, T., Fujihara, S., Katsura, T., Yamamoto, K., Inubushi, T., Tanimoto, K., Tanaka, E., 2010 Nov. Effects of low-intensity pulsed ultrasound on the expression and activity of hyaluronan synthase and hyaluronidase in IL-1β-stimulated synovial cells. Ann Biomed Eng. 38 (11), 3363–3370. https://doi.org/10.1007/s10439-010-0104-5.

Oga, S., Goto, K., Sakamoto, J., Honda, Y., Sasaki, R., Ishikawa, K., Kataoka, H., Nakano, J., Origuchi, T., Okita, M., 2020 May. Mechanisms underlying immobilization-induced muscle pain in rats. Muscle Nerve. 61 (5), 662–670. https://doi.org/10.1002/mus.26840.

Park, S.Y., Lee, S.W., Lee, S.Y., Hong, K.W., Bae, S.S., Kim, K., Kim, C.D., 2017 Sep. SIRT1/Adenosine Monophosphate-Activated Protein Kinase α Signaling Enhances Macrophage Polarization to an Anti-inflammatory Phenotype in Rheumatoid Arthritis. Front Immunol. 15 (8), 1135. https://doi.org/10.3389/fimmu.2017.01135.

Rosenberger, D.C., Pogatzki-Zahn, E.M., 2022 May. Chronic post-surgical pain - update on incidence, risk factors and preventive treatment options. BJA Educ. 22 (5), 190–196. https://doi.org/10.1016/j.bjae.2021.11.008.

Rosim, G.C., Barbieri, C.H., Lanças, F.M., Mazzer, N., 2005 Mar. Diclofenac phonophoresis in human volunteers. Ultrasound Med Biol. 31 (3), 337–343. https://doi.org/10.1016/j.ultrasmedbio.2004.11.012.

Ruffino, J.S., Davies, N.A., Morris, K., Ludgate, M., Zhang, L., Webb, R., Thomas, A.W., 2016 Sep. Moderate-intensity exercise alters markers of alternative activation in circulating monocytes in females: a putative role for PPARγ. Eur J Appl Physiol. 116 (9), 1671–1682. https://doi.org/10.1007/s00421-016-3414-y.

Sasaki, R., Sakamoto, J., Kondo, Y., Oga, S., Takeshita, I., Honda, Y., Kataoka, H., Origuchi, T., Okita, M., 2021. Effects of cryotherapy applied at different temperatures on inflammatory pain during the acute phase of arthritis in rats. Phys Ther 101 (2). https://doi.org/10.1093/ptj/pzaa211.

Sekiguchi, M., Shirasaka, M., Konno, S., Kikuchi, S., 2008. Analgesic effect of percutaneously absorbed non-steroidal anti-inflammatory drugs: an experimental study in a rat acute inflammation model. BMC Musculoskelet Disord. 9, 15. https://doi.org/10.1186/1471-2474-9-15.

Sugiyama, Y., Iida, H., Amaya, F., Matsuo, K., Matsuoka, Y., Kojima, K., Matsuno, F., Hamaguchi, T., Iseki, M., Yamaguchi, K., Takahashi, Y., Hara, A., Sugasawa, Y., Kawamata, M., Tanaka, S., Inagaki, Y., Otsuki, A., Yamazaki, M., Ito, H., 2018 Jun. Prevalence of chronic postsurgical pain after thoracotomy and total knee arthroplasty: a retrospective multicenter study in Japan (Japanese Study Group of Subacute Postoperative Pain). J Anesth. 32 (3), 434–438. https://doi.org/10.1007/s00540-018-2481-0.

Tak, P.P., Bresnihan, B., 2000 Dec. The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis. Arthritis Rheum. 43 (12), 2619–2633. https://doi.org/10.1002/1529-0131(200012)43:12<2619::AID-ANR1>3.0.CO;2-V.

Takano, S., Uchida, K., Miyagi, M., Inoue, G., Aikawa, J., Fujimaki, H., Minatani, A., Sato, M., Iwabuchi, K., Takaso, M., 2016 Jan. Synovial macrophage-derived IL-1β regulates the calcitonin receptor in osteoarthritic mice. Clin Exp Immunol. 183 (1), 143–149. https://doi.org/10.1111/cei.12712.

Takeuchi, R., Ryo, A., Komitsu, N., Mikuni-Takagaki, Y., Fukui, A., Takagi, Y., Shiraishi, T., Morishita, S., Yamazaki, Y., Kumagai, K., Aoki, I., Saito, T., 2008. Low-intensity pulsed ultrasound activates the phosphatidylinositol 3 kinase/Akt pathway and stimulates the growth of chondrocytes in three-dimensional cultures: a basic science study. Arthritis Res Ther. 10 (4), R77. https://doi.org/10.1186/ar2451.

Tidball, J.G., Dorshkind, K., Wehling-Henricks, M., 2014 Mar. Shared signaling systems in myeloid cell-mediated muscle regeneration. Development. 141 (6), 1184–1196. https://doi.org/10.1242/dev.098285.

Ulubay, M., Yurt, K.K., Kaplan, A.A., Atilla, M.K., 2018 Jan. The use of diclofenac sodium in urological practice: A structural and neurochemical based review. J Chem Neuroanat. 87, 32–36. https://doi.org/10.1016/j.jchemneu.2017.02.005.

van Driel, M.E.C., van Dijk, J.F.M., Baart, S.J., Meissner, W., Huygen, F.J.P.M., Rijsdijk, M., 2022 Sep. Development and validation of a multivariable prediction model for early prediction of chronic postsurgical pain in adults: a prospective cohort study. Br J Anaesth. 129 (3), 407–415. https://doi.org/10.1016/j.bja.2022.04.030.

Woolf, C.J., 2011 Mar. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 152 (3 Suppl), S2–S15. https://doi.org/10.1016/j.pain.2010.09.030.

Yang, J.H., Kim, T.Y., Lee, J.H., Yoon, S.W., Yang, K.H., Shin, S.C., 2008 Apr. Anti-hyperalgesic and anti-inflammatory effects of ketorolac tromethamine gel using pulsed ultrasound in inflamed rats. Arch Pharm Res. 31 (4), 511–517. https://doi.org/10.1007/s12272-001-1186-5.

Zhao, Y.Z., Du, L.N., Lu, C.T., Jin, Y.G., Ge, S.P., 2013. Potential and problems in ultrasound-responsive drug delivery systems. Int J Nanomedicine. 8, 1621–1633.

Xuan, W., Qu, Q., Zheng, B., Xiong, S., Fan, G.H., 2015. Jan. The chemotaxis of M1 and M2 macrophages is regulated by different chemokines. J Leukoc Biol 97 (1), 61–69. https://doi.org/10.1189/jlb.1A0314-170R.