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Reduction of Edema and Pain in Transcutaneous Electrical Nerve Stimulation Treated-Arthritic Rat

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Transcutaneous electrical nerve stimulation (TENS) has been used to reduce pain or improve motor function in musculoskeletal and neurological disorders in the clinic. Although some studies have suggested electrotherapy as an intervention for edema, the effects and mechanisms of TENS on inflammation-induced edema remain unclear. Thus, we aimed to investigate the effects of TENS on arthritic pain with edema. 1% carrageenan was injected into the right tibiofemoral joint of 69 male Sprague–Dawley rats (200-250 g). After the development of arthritic pain, low-frequency (4-Hz, Low-TENS, n = 25) and high-frequency (100-Hz, High-TENS, n = 25) TENS with sub-motor threshold or placebo-TENS (n = 19) was applied for 20-min to medio-lateral part of the ipsilateral side. Weight bearing and knee-bend tests were used to assess pain-like behaviors. Also, we examined the size of edema and measured tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels in the synovium by western blot. Eight rats in each of the two TENS groups were injected with Naloxone. Edema was reduced in the low- and high-frequency TENS groups at 6-h. TENS-treated rats showed reduced pain in the knee-bend test at 6-h. We observed decreased weight load shifts on the ipsilateral side in TENS groups. Naloxone reduced these effects. TNF-α and IL-1β expression decreased in the synovial membrane at 6-h. These results suggest that low- and high-frequency TENS have acutely positive effects on inflammatory edema, with the management of arthritic pain and reduction in pro-inflammatory mediators. Therefore, Low-TENS and High-TENS may be useful in treating acute inflammatory pain and edema.

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Introduction

Inflammation is a physiological response to traumatic injury, infection, toxic compounds, irradiation, arthritis, or surgical procedures. It triggers the process of removing harmful stimuli from the body and healing damaged tissue to protect itself (Amaya et al. 2013). The main symptoms of inflammation include redness, swelling, heat, pain, and dysfunction (Takeuchi and Akira 2010). In arthritis, pathophysiological changes such as synovial hyperplasia, cartilage damage, osteophyte formation, and bone erosion are induced (Neugebauer et al. 2007). Inflammation can be classified as acute or chronic. Acute inflammation occurs frequently and, if left untreated, leads to chronic inflammation (Dieppe and Lohmander 2005). In previous animal studies, carrageenan was injected into the joint cavity to induce acute inflammation in joint tissues, which resulted in acute inflammatory reactions such as synovial fluid exudation (Neugebauer et al. 2007). In addition, pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) are released from the inflamed tissue, resulting in sensitization of peripheral nerves (LeGrand et al. 2001). Such physiological changes increase vascular permeability and cause edema, which makes movement difficult and increases mechanical pressure due to dilatation, which causes pain and impedes movement (Luna et al. 2019). Thus, it is necessary for the intervention of acute inflammatory response.

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Electrical stimulation (ES) is clinically used to enhance and maintain muscle strength, prevent atrophy, improve motor function, manage spasticity, and control pain (Bergquist et al. 2011). It is non-invasive, easy to apply, and has few side effects. Although ES has been proposed for the management of inflammation and edema, its effects and mechanisms remain unclear. Some studies have suggested that ES is effective for edema intervention in patients with lymphedema and traumatic injury (Thornton et al. 1998; Choi and Lee 2016), whereas other studies have reported no positive effects on reducing inflammation and edema induced by intradermal injection of serotonin into the paw and carrageenan into the knee joint (Vance et al. 2007; Santos et al. 2013).

Among the various ES methods, transcutaneous electrical nerve stimulation (TENS) is the most used intervention in clinical and rehabilitation centers for reducing pain and improving motor function (Sluka et al. 2000). Several previous studies have reported the analgesic effects of different mechanisms according to the frequency stimulation intensity or pulse duration of TENS (Sluka and Walsh 2003). Applying low- (4 Hz) and high- (100 Hz) frequency TENS to the acute arthritic knee joint showed analgesic effects by enhancing the activation of μ-opioid and δ-opioid receptors in the spinal cord, respectively (DeSantana et al. 2009). Another study reported that applying high-frequency TENS to rats with acute arthritis reduced the release of excitatory neurotransmitters, such as aspartate and glutamate, in the dorsal horn of the spinal cord (Sluka et al. 2005). In addition, a study that analyzed 169 reviews and 49 meta-analyses found that TENS effectively reduced chronic musculoskeletal pain and labor pain in humans compared to controls, as well as reduced postoperative analgesic consumption, and recommended it as a treatment option for pain intervention (Paley et al. 2021).

While TENS has proven analgesic effects in humans and animals that can effectively reduce various musculoskeletal disorders and inflammatory pain, the effects of TENS on edema are unclear and under-researched. In a plantar inflammation animal model induced by carrageenan and 5-hydroxytryptamine, in which low- and high-frequency TENS were applied as a single application, it was confirmed that the pain decreased at a specific time but had no significant effect on edema (Santos et al. 2013). However, a clinical study on lymphedema in humans confirmed that four weeks of TENS application reduced edema (Choi and Lee 2016). These differences in results may be due to differences in the models used in the studies, the duration of the intervention, the people and animals involved, and the body parts affected by edema, and it is essential to clarify the effects of TENS on the disease and cause of edema.

Therefore, the present study aims to investigate the effects of low- and high-frequency TENS application on pain behavior and edema using changes in circumference and mediolateral distance of the knee in a carrageenan-induced acute joint inflammation model. Additionally, we aim to assess the alterations in TNF-α and IL-1β levels in the synovial membrane. This investigation aims to determine whether TENS has an impact on swelling and pain.

Methods

Animals

Male Sprague–Dawley rats (n = 72, 200-250 g; Orient Bio Inc., Seongnam, Korea) were used as experimental animals. After inducing acute inflammation in the right knee joint in 69 rats, 25 each received low-frequency TENS (Low-TENS group) or high-frequency TENS (High-TENS group), and 19 received placebo stimulation (Placebo-TENS group). In each of the two TENS groups, seven rats were used to assess pain behavior, seven rats were used to determine changes in edema, eight rats were treated with Naloxone, and three rats were used for western blot. The Placebo TENS group consisted of 8 rats, each used to measure pain behavior and edema, and three rats used to analyze synovial membrane tissue. Three naive rats did not receive any intervention and were used to confirm the normal condition of the synovial membrane.

The rats were housed in a temperature- and light-controlled room (22-25°C, 12 h light/dark cycle) and were allowed to acclimate to the housing conditions for 1 week before experimentation. Food (5L79; PMI Nutrition, St. Louis, MO, USA) and water were provided ad libitum. This study was approved by the Institutional Animal Care and Use Committee of Korea University (approval number: KOREA-2021-0140).

Carrageenan-induced arthritis model

To induce acute arthritis, 1% lambda-carrageenan (50 μL, Sigma-Aldrich, Burlington, MA, USA) was injected into the tibiofemoral joint of the right knee joint of each rat under inhalation anesthesia consisting of isoflurane (0.5-2%). The injected leg was manually flexed and extended for approximately 2 min to distribute the knee joint cavity.

TENS

Rats received TENS under brief isoflurane anesthesia (0.5-2%), which can exhibit an avoidance response to pain-inducing stimuli. TENS was applied 4.5 hours after the carrageenan injection. Electrodes were placed parallel to the medial and lateral sides of the inflamed knees. Both low-frequency (4 Hz) and high-frequency (100 Hz) TENS were applied at the sub-motor threshold intensity for 20 min. In contrast, Placebo-TENS used the same method, but no actual ES was applied.

Naloxone application

The anti-nociceptive effect of TENS was blocked by intraperitoneal pretreatment with Naloxone (μ-opioid receptor antagonist, 2 mg/kg) before low- and high-frequency TENS application. An evaluation was performed 6 hours after carrageenan injection using two pain behavioral tests.

Edema measurements

Carrageenan-induced arthritis is always accompanied by knee joint edema. The size of the edema was assessed by measuring the knee circumference using a string and knee diameter, that is, the distance from the medial to the lateral side of the knee, using a Vernier caliper at different time points (before, 4, 6, 8, 10, and 24 hours). Measurements were performed under brief isoflurane anesthesia (0.5-2%).

Behavioral studies

Weight-bearing test: Weight loaded onto the side of an inflamed joint can cause pain. Using a dynamic weight load device developed in our laboratory, the weight load on the limbs of the rats was measured while walking freely (Lee et al. 2007). The bottom of the path was equipped with a load cell sensor (DACELL, CB1-K2, Cheongwon, Korea), and output signals were transmitted to a digital amplifier (DACELL, DN-AM 300) for amplification and filtering. The signal was digitized using an analog-digital converter (DACELL, 1716) and plotted as time-weight curves on a personal computer (WBT, Korea University, Seoul, Korea). The test was repeated three to four times to obtain at least eight time-weight curves for a given limb. This was observed and noted during testing. We confirmed the time course of the weight load test (before, 4, 6, 8, 10, and 24 hours after carrageenan injection). The weight load values of the affected leg showed the most notable decrease at 4 hours after carrageenan injection.

Knee-bend test: The knee-bend test was performed as previously described (Ferreira-Gomes et al. 2008). The test calculates the squeak or struggle reactions in response to flexions and extensions of the knee joint within its limits of movement. Each trial consisted of 5 flexions and 5 extensions of the knee joint, and scores were determined based on the response type for each joint movement, similar to previous research. The sum of the calculated reactions, giving a maximum value of 20, represents the knee-bend score, representing nociception due to the animal's movement. The contralateral knee was always tested first to avoid an increase in the contralateral score due to the manipulation of the injected knee. We confirmed the time course of the knee-bend test (before, 4, 6, 8, 10, and 24 hours after carrageenan injection). The anti-nociceptive effect of TENS was blocked by intraperitoneal pretreatment with Naloxone (μ-opioid receptor antagonist, 2 mg/kg) before low- and high-frequency TENS application. The knee-bend test was measured at 6 hours.

Changes in protein expression by western blot

Rats were sacrificed, and synovial membrane samples were extracted for measurement of pro-inflammatory mediators (TNF-α and IL-1β) at 6 hours. The synovial membranes were harvested from the anterior part of the knee joint on the normal and inflamed sides. After removal, the synovial membrane was placed in 200 μL cell lysis buffer [50 mM Tris-HCl, 5 mM ethylenediaminetetraacetic acid, 150 mM sodium chloride (NaCl), and 1% nonyl phenoxypolyethoxylethanol] and incubated for 30 min. After centrifugation for 20 min, the total protein in the separated supernatants was quantified using the Bradford method with bovine serum albumin. The protein (30 μg) was separated on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to a nitrocellulose membrane (Whatman Protran, 0.45 μm, Thermo-Fisher Scientific, Waltham, MA, USA) at 55 V for 4 hours using a Mini-PROTEAN 3 (Bio-Rad, Hercules, CA, USA) device. The membranes were blocked with 5% nonfat milk in TBST (Tris buffered saline with Tween 20; 0.5% Tween-20 in 20 mM Tris, 137 mM NaCl) for 1 hour at room temperature and probed with antibodies against IL-1β (1:1,000, ab9787, Abcam, Cambridge, United Kingdom) and TNF-α (1:1,000, ab66579, Abcam) overnight at 4°C, with rocking. The membranes were washed thrice for 15 min each in TBST. Goat anti-rabbit or anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibody (1:2,000-1:5,000, sc-2004, sc-2031, Santa Cruz, Santa Cruz, CA, USA) was then added to a 5% blocking buffer for 2 hours at room temperature with rocking. The protein bands were detected by chemiluminescence (Pierce®EC, Thermo-Fisher Scientific) on medical X-ray film (CP-BU, Agfa, Mortsel, Belgium), with β-actin (1:1,000, sc-130656, Santa Cruz, USA) used as a loading control. The densities of specific bands were measured using ImageJ software and normalized against the loading control.

Data analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences software (version 23.0; SPSS, Inc., Chicago, IL, USA). To compare changes in edema, knee-bend test, and weight-bearing associated with inflammation and pain, a two-way analysis of variance (ANOVA) was used to compare variances at different time points between groups. One-way ANOVA with post-hoc Tukey analysis was used to compare variances in rearing count and duration between the placebo, Naloxone-injected, and Naloxone-non-injected groups. The level of significance was set at $p<0.05$.

Results

TENS application decreases edema of inflamed knee joints

In both edema measurement methods, the size of edema of the knee joint persisted until 24 hours in the Placebo-TENS group (Fig. 1). Low- and high-frequency TENS application showed a significant decrease in edema only at 6 hours in the mediolateral distance measurement compared with Placebo-TENS ($p < 0.05$; Fig. 1A). Circumference measurements showed that both methods significantly reduced edema at 6 hours compared with Placebo-TENS, and only high-frequency TENS stimulation showed a significant change compared with Placebo-TENS stimulation at 8 hours ($p < 0.05$; Fig. 1B). No significant

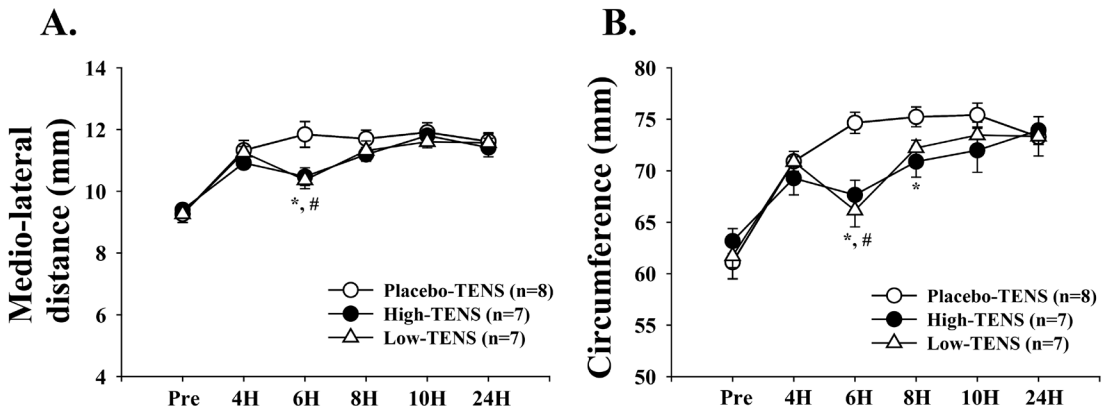

Fig. 1. Changes in the medio-lateral distance (A) and circumference (B) of edema by application of Low-TENS, High-TENS, and Placebo-TENS from 4 hours to 24 hours after injection of 1% carrageenan.
Induction of edema was shown in the three groups starting at 4 hours after carrageenan injection and was maintained until 24 hours later. Edema was evaluated by measuring (A) the medio-lateral distance and (B) the circumference of the knee joint on the ipsilateral side. Data are presented by mean ± standard deviation. * means a significant difference between High-TENS and Placebo-TENS. # means a significant difference between Low-TENS and Placebo-TENS.

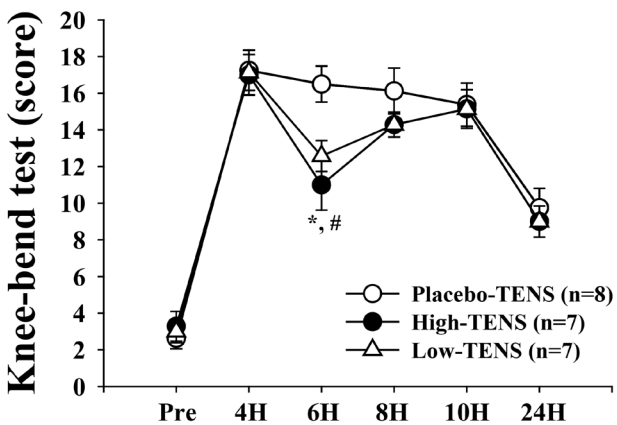

Fig. 2. Changes in the knee-bend score by application of Low-TENS, High-TENS, and Placebo-TENS from 4 hours to 24 hours after injection of 1% carrageenan.
Increases in pain behavior were significant at 4 hours in all three groups, and both TENS groups reduced pain compared to the Placebo-TENS at 6 hours. Data are presented by mean ± standard deviation. * means a significant difference between High-TENS and Placebo-TENS. # means a significant difference between Low-TENS and Placebo-TENS.

differences between the Low-TENS and High-TENS groups exists at any time point ($p > 0.05$).

TENS application reversed inflamed knee joint-related pain behavior

In the knee-bend test, the application of low- and high-frequency TENS resulted in a significant decrease at 6 hours compared with Placebo-TENS ($p < 0.05$; Fig. 2). In addition, both methods showed an improvement in pain behavior at 8 hours, but the improvement was not significant ($p > 0.05$).

The weight-bearing test showed a pattern similar to that of the knee-bend test. Both Low-TENS and High-TENS showed a significant increase in weight-bearing at 6 hours on the ipsilateral side ($p < 0.05$; Fig. 3A). There was also a tendency for an increase at 8 hours, but this difference was not statistically significant. On the contralateral side, there was no significant difference between Placebo-TENS and the application of the two TENS interventions at any measurement time point (Fig. 3B).

Behavioral outcomes of Naloxone application with TENS

In the weight-bearing test, the application of Naloxone with low- and high-frequency TENS did not show statistical significance compared to the Placebo-TENS. However, in comparison to the group that received only low- and high-TENS without Naloxone treatment, the Naloxone-treated rats tended to increase pain behavior (Fig. 4A). In the knee-bend test, Naloxone-treated rats in the High-TENS showed statistical significance when compared to the Placebo-TENS ($p < 0.05$; Fig. 4B).

TENS application decreased the expression level of inflammatory mediators TNF-α and IL-1β in the synovial membrane

As edema and pain behavior were reduced at 6 hours, we observed changes in TNF-α and IL-1β in the synovial membrane of the inflamed knee joint (Fig. 5). In the Placebo-TENS group, two pro-inflammatory cytokines were significantly increased, whereas low- and high-frequency TENS application decreased the expression of TNF-α and IL-1β (Fig. 5).

Discussion

This study confirmed that low- and high-frequency TENS applications have effectively improved the size of the edema and pain caused by acute inflammation. In the knee, the size of edema was reduced in both medial-lateral

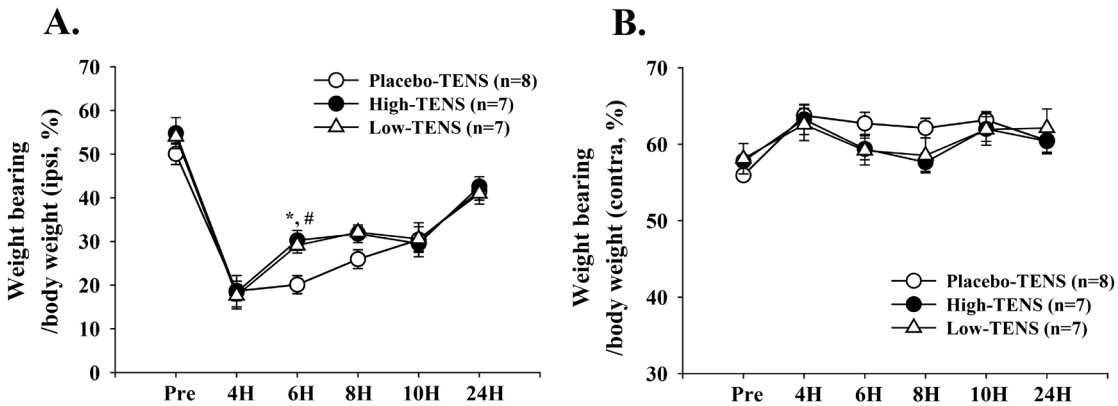

Fig. 3. Changes in the weight bearing of the ipsilateral side (A) and contralateral side (B) by application of Low-TENS, High-TENS, and Placebo-TENS from 4 hours to 24 hours after injection of 1% carrageenan.
There were no significant differences in the contralateral side among the three groups, while Low-TENS and High-TENS showed significant improvement compared to placebo-TENS at 6 hours on the ipsilateral side. Data are presented by mean ± standard deviation. * means a significant difference between High-TENS and Placebo-TENS. # means a significant difference between Low-TENS and Placebo-TENS.

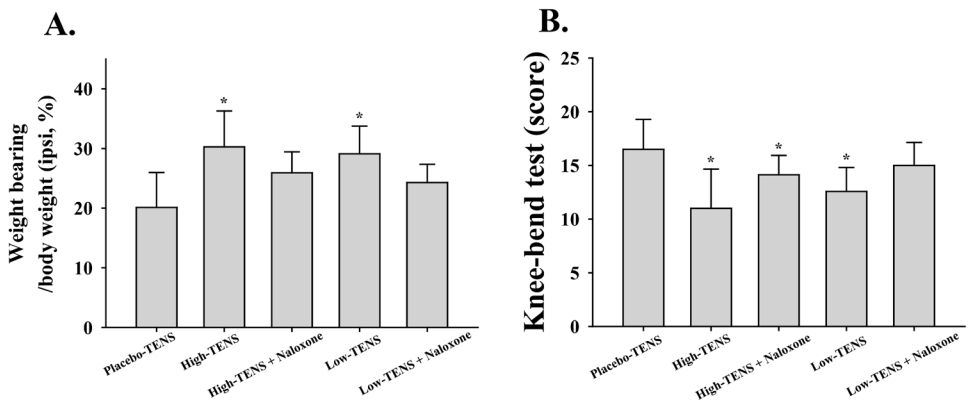

Fig. 4. Changes in the weight bearing (A) and knee-bend score (B) of the ipsilateral side with/without the intraperitoneal injection of Naloxone.
An evaluation was performed 6 hours after carrageenan injection and showed that the analgesic effects of Low-TENS and High-TENS tended to be reduced by naloxone application. Data are presented by mean ± standard deviation. * indicates a significant difference compared to Placebo-TENS.

distance and circumference measurements. In addition, both TENS applications improved primary pain behavior related to tissue inflammation in the knee-bend test, and they significantly increased the degree of weight-bearing on the involved side in the weight-bearing test. Furthermore, TENS applied to the peripheral tissue significantly reduced inflammatory cytokines such as TNF-α and IL-1β in the synovial membrane.

ES has been mainly used to control pain caused by damage to the musculoskeletal and nervous systems in experimental medical research and clinics. In acute arthritis, symptoms, such as pain and swelling, are induced by a rapid increase in inflammatory substances in the accompanying tissues, and the intervention of inflammatory substances can lead to the management and resolution of these symptoms. The effects of ES using TENS on pain have been reported, whereas reports on inflammation and edema are insufficient or unclear. Contrary to the results of this study, Sluka et al. (1998) reported that low- and high-frequency TENS effectively reduced pain caused by knee arthritis but did not significantly affect edema. They injected 3% kaolin and 3% carrageenan to induce arthritis, which led to severe inflammation, and severe pain persisted for 24 hours. On the other hand, we used a model in which mild to moderate inflammation was induced by the injection of 1% carrageenan, and almost all pain was recovered within 24 hours. Based on the differences between the results of this study and those of Sluka's study, it is speculated that the anti-inflammatory effect of TENS differs according to the severity of inflammation (Sluka et al. 1998). Sluka et al. (1998) measured edema by circumference and found that it tended to increase from approxi-

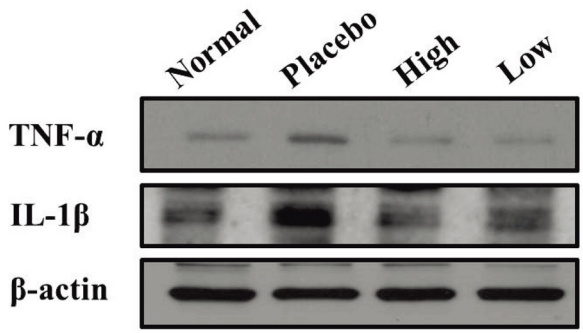

Fig. 5. Changes in TNF- α and IL-1β protein expressions in synovial membrane tissue at 6 hours.
The knee joint injected with carrageenan 1% showed changes in TNF-α and IL-1β expression in the synovial membrane tissue (Placebo-TENS). Low-TENS and High-TENS showed significantly decreased TNF-α and IL-1β expression in the synovial membrane at 6 hours.

mately 62-65 mm at baseline to approximately 74-81 mm at 4H and 75-84 mm at 8H, whereas the edema in this study was 61-63 mm at baseline, 69-71 mm at 4H, and 70-75 mm at 8H using the same measurements. This suggests that the drug concentration used in Sluka's study caused severe inflammation, while the 1% in this study caused moderate inflammation. In this study, the level of edema was measured in two ways: circumference and mediolateral distance. Both methods showed a similar tendency to induce edema, but interestingly, when comparing the ratio (%), the mediolateral distance was greater (4H-Pre: mediolateral distance 16-21% vs. circumference 9-16%). We recommend that future studies measuring edema take our results into account.

In the study by Sluka et al. (1998), low- and high-frequency TENS tended not to reduce edema over time compared to control. However, in our study, the placebo-TENS group increased slightly in the mediolateral distance from 4H 11.2 mm to 6H 11.7 mm, but low-frequency TENS applied at 4.5H reduced it by approximately 7.9% from 4H 11.2 mm to 6H 10.4 mm, and high-frequency TENS reduced it by approximately 4% from 4H 10.9 mm to 6H 10.5 mm. Therefore, TENS is more effective in reducing edema caused by moderate inflammation than that caused by severe inflammation, and the effect of TENS on edema is thought to differ depending on the method of inducing inflammation.

However, in contrast to our study, a previous study injected 250 μg/0.1 ml carrageenan to induce inflammation and reported that TENS application did not effectively mediate edema despite the inflammation induced by the low concentration (Resende et al. 2004). These differences in results are likely due to differences in the site of inflammation, the method of inducing inflammation, and the application of TENS between the studies. We injected a higher concentration of carrageenan (1 mg/0.1 ml) into the knee joint space, while Resende et al. (2004) injected a lower concentration (250 μg/0.1 ml) subcutaneously to induce inflammation. While the subcutaneous area has relatively little space for carrageenan to diffuse and only soft tissues such as muscle and skin, many more structures within the knee joint include synovial fluid, ligaments, and joint capsule. Therefore, the difference in the composition of these tissues and the amount of space for inflammatory substances to spread may have contributed to the discrepancy between the studies. Also, the knee joint is a larger structure than the hind paw, which may have contributed to the difference in results.

Similar to previous studies, this study confirmed that low- and high-frequency TENS improved pain behavior (Sluka et al. 1998; Gopalkrishnan and Sluka 2000). The knee arthritis model injected with carrageenan is widely used to study acute arthritis and various concentrations of carrageenan are used depending on the researcher. In a knee arthritis model using 3% carrageenan, high-frequency TENS reduced pain response to mechanical stimuli in the paw for up to 8 hours after intervention (Gopalkrishnan and Sluka 2000). In addition, in the same model, low- and high-frequency TENS significantly reduced thermal pain behavior from the paw to 8-12 hours (Sluka et al. 1998). On the other hand, we showed a significant decrease after 2 hours in the knee-bend and weight-bearing tests following the two TENS applications. The difference in these results may be due to the dose difference of the drug used to induce acute inflammation, but it is also presumed to be due to the difference in the site where the pain behavior was measured. While previous studies measured secondary pain induced in the paw by knee arthritis, our method measured primary pain behavior by knee arthritis. Based on these results, it is speculated that TENS can be effectively used for the intervention of primary and secondary pain caused by acute knee arthritis and propose the application of TENS considering the severity of inflammation.

To investigate the relationship between edema and pain, we injected Naloxone, which reduces the analgesic effects of TENS, and confirmed that the effects of low- and high-frequency TENS on primary pain were reduced (Fig. 4). Interestingly, in the knee-bend test, pain behavior was reduced due to the maintenance of the analgesic effect in high-frequency TENS treated with Naloxone compared with Placebo-TENS. These results may be due to physiological responses such as gamma-aminobutyric acid release in the spinal cord and other neural structures by TENS (Maeda et al. 2007). In addition, edema is a factor that can affect hypersensitivity and pain response, and it is assumed that the reduction of edema by TENS influenced pain reduction in this study (Lariviere et al. 2005). The reduction in edema can alleviate the restriction of joint movement, along with the reduction of mechanical stress on the inflamed tissue. In this study, this correlation can be inferred from the fact that the measurement time points for edema and pain reduction were almost identical.

According to previous studies, the reduction in inflam-

mation can be observed through changes in the levels of inflammatory cytokines, which can reduce inflammatory substances by mechanical or ES (Chen et al. 2015). Among several cytokines, it has been reported that TNF-α and IL1-β are regulated by TENS. TENS inhibited the upregulation of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and substance P in the model for skin and muscle incision and retraction. In the neuronal cell model, TENS-like stimulation reduced TNF-α, IL-1β, and IL-6. It has also been reported that TENS inhibits cytokines and is effective for wound healing (Sovak and Budgell 2017). Reduction of TNF-α and IL-1β through TENS mediation in skin and nerve cells means that the effects of TENS are similar regardless of tissue. Reports show that high-frequency ES other than TENS reduces cytokine levels in the synovial membrane, suppresses pain, and reduces macrophages, suggesting that the inhibition of cytokines plays a significant role in reducing knee pain and inflammation.

The present study investigated the effect of low- and high-frequency TENS application on the reduction of pain, edema, and inflammatory substances. Contrary to previous reports, the concentration of carrageenan applied in the current study is inferred to be appropriate for confirming the effects of TENS. Furthermore, it is expected that there will be a more pronounced reduction in edema when repeated TENS is applied. If the mechanism by which inflammatory substances are reduced in the synovial membrane is identified, the effect of TENS on edema will be verified.

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Author Contributions

Conception and design: H.R.S. and H.C.; Supervision: H.C.; Investigation and methodology: H.R.S.; Measurement: H.R.S. and H.C.; Formal analysis: H.R.S.; Writing-original draft preparation: H.R.S.; Writing-review and editing: H.C.

Conflict of Interest

The authors declare conflict of interest.

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