Anti-Hyperalgesic Effects of Meloxicam Hydrogel via Phonophoresis in Acute Inflammation in Rats; Comparing Systemic and Topical Application

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Abstract – The aim of this study was to determine if a meloxicam hydrogel could be administered in vivo via phonophoretic transdermal delivery using pulsed ultrasound by examining its anti-hyperalgesic effects in a rat carrageenan inflammation model. Carrageenan (1%) was injected into the plantar surface of the right hindpaw, and meloxicam hydrogel was administered via phonophoretic transdermal delivery. Changes in the mechanical and thermal hyperalgesia, as well as swelling, showed that phonophoretic delivery of meloxicam exhibited significantly better anti-hyperalgesic and anti-inflammatory effects than pulsed ultrasound. Topical and systemic application of meloxicam hydrogel using phonophoresis showed similar anti-hyperalgesic effects. These findings suggest that the transdermal administration of a meloxicam hydrogel using phonophoresis by pulsed ultrasound might be useful for treating acute inflammation.

Keywords: Meloxicam hydrogel, Phonophoretic transdermal delivery, Anti-hyperalgesia, Systemic application, Topical application, Acute inflammation

INTRODUCTION

Meloxicam, a non-steroidal anti-inflammatory drugs (NSAIDs) with both anti-inflammatory and anti-noceptive actions, is widely used in clinical practice (Todd and Sorkin, 1988; Mishra and Vijaya Kumar, 2006). Delivering NSAIDs through the skin for either local or systemic treatment has been investigated (Rolf *et al.*, 1999; Cagnie *et al.*, 2003). Local and systemic effects that can be distinguished by determining the local tissue drug concentrations and plasma levels (Cagnie *et al.*, 2003).

The skin provides potential for non-invasive drug delivery (Foldvari, 2000). However, the low permeability of skin due to the barrier properties of the stratum corneum limits the passive transdermal approach of most drugs (Median *et al.*, 1995; Fang *et al.*, 2001). Many studies have focused on methods for overcoming the barrier properties of the stratum corneum and for promoting drug permeability using chemical agents or physical modalities (Jadoul *et al.*, 1999).

Phonophoresis (Tyle and Agrawala, 1989), which uses ultrasound with a chemical agent, is often applied for transdermal delivery in clinics with iontophoresis using direct current (Tyle and Agrawala, 1989; Riviere and Heit, 1997). Phonophoresis has been used successfully to deliver anti-inflammatory medication to inflamed subcutaneous tissue (Bare *et al.*, 1996; Cagnie *et al.*, 2003; Yang *et al.*, 2005; Yang *et al.*, 2008). The major advantages are the introduction of medication to a local area without invasion of the skin and the synergistic interaction of ultrasound and drugs (Wells, 1977). Phonophoresis with NSAIDs is commonly used to treat inflamed tissues (Byl, 1995; Hsieh, 2006). The direct peripheral anti-hyperalgesic effects of ultrasound and phonophoresis with NSAIDs in treating injured tissue have been widely investigated (Guffey and Knaust, 1997), but the possible effects of ultrasound and phonophoresis with NSAIDs on the central modulation of inflammatory nociception are still unclear (Hsieh, 2006).

This study examined the effectiveness of meloxicam using phonophoretic transdermal delivery by determining its anti-hyperalgesic effects in a rat carrageenan inflammation model. We compared the anti-hyperalgesia and anti-edema effects of meloxicam hydrogel, as well as differences in top-

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ical and systemic administration.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (253 ± 1.23 g) were purchased from the Daehan Laboratory Animal Research Co. (Choongbug, Korea), and given access to normal standard chow diet (Jae Il Chow, Korea) and tap water *ad libitum*. The animals were housed in laminar flow cages, four or five rats to a cage, and were maintained at $22 \pm 1^\circ\text{C}$, relative humidity of 40–60%, and a 12-h light-dark cycle. The animals were allowed to acclimatize for at least one week before the experiments. The experiments were performed in accordance with the “ethical standards set by the Helsinki Declaration of the World Medical Association” in 1964, revised in 2004.

Preparation of meloxicam hydrogel

One gram of carbopol 940 was dissolved in 40 ml of distilled water. Then, 20 ml of propylene glycol, 5 ml of Labrafil and 30 ml of ethanol containing meloxicam was mixed with constant stirring and adjusted to pH 7.0 using triethanolamine. Water was added to the solution to make a final volume of 100 ml. The prepared gels were stable at 50°C for 6 months.

Induction of hyperalgesia

Paw hyperalgesia was induced by injecting 1% carrageenan as described by Levy (1969) and Schrier *et al.* (1987). Prior to drug treatment, a 1% carrageenan suspension (0.1 ml) was injected into the subplantar area of the right hindpaw and the withdrawal latency was measured. The animals were tested before injecting carrageenan (Dowdall *et al.*, 2005) as the baseline (time 0), and at 3, 6, 9 h after the carrageenan injection. The animals were returned to their cage until they were tested.

Paw swelling test

The extent of paw swelling was determined by measuring the change in the paw volume. The paw swelling was measured on the right foot using a digimatic caliper (Mitytoyo, Japan), before and at 3, 6, and 9 h after carrageenan injection. Their mean values were calculated (the average of three determinations per group).

Mechanical pain threshold

For the measurement of mechanical pain threshold, rats were placed in transparent lucite cubicles that allow minimal movement on an elevated meshed platform. The von Frey

monofilaments (Touch - Test Sensory Evaluation, North Coast Medical, USA) were applied at right angles to the plantar surface of the paw starting with the lowest bending force and then increasing with logarithmically incremental stiffness (1.4, 2, 4, 6, 8, 10, 15, 26 g) to calculate the 50% probability thresholds for mechanical paw withdrawal. Mechanical pain threshold, i.e., the lowest bending force at which the animal lifts its paw off the meshed platform, was noted. Their mean values were calculated (the average of three determinations per group).

Thermal pain threshold

To determine the paw withdrawal latency, animals were placed on a hot plate (DJM, Korea) with a surface temperature of 30°C for 10 minutes. The surface temperature of the hot plate was increased to $50 \pm 0.2^\circ\text{C}$ and the paw withdrawal latency time was determined. The time for paw withdrawal was counted from the time the heat was applied to the subplantar area of the right hindpaw showing hyperalgesia. Any lifting associated with normal locomotion was excluded. The lifting of the right paw could be explained by a combination of factors including thermal exposure and the animal adjusting its weight onto the uninjured paw (Dowdall *et al.*, 2005). Their mean values were calculated (the average of three determinations per group).

Drug application

Primary experiment: The changes in anti-hyperalgesia and anti-swelling effects by dose of meloxicam were determined. The rats were divided into four groups of six each. The group I (control group) did not receive any treatment. The meloxicam hydrogel (0.5%) was used on the abdomen for group II, 1% for group III, and 2% for group IV.

Secondary experiment: We then compared the effects of topical and systemic meloxicam hydrogel. The rats were divided into five groups of six each. The group I (control group) did not receive any treatment. The meloxicam hydrogel (1%) with phonophoresis was used on the abdomen area in group II or on the paw area in the group III. In groups II and III, an ultrasound transducer with 0.8 cm^2 of effective radiation area (ERA) and 6.0 max beam non-uniformity ratio (BNR) which indicates the relationship between the spatial peak intensity and spatial average intensity (Bare *et al.*, 1996). The treatment parameter was a pulsed mode, its duty cycle was 20%, the ultrasound frequency was 1 MHz, the treatment intensity was 1.0 W/cm^2 , the treatment area was two-fold wider than the ERA, and the treatment time was 5 min. In groups II and III, approximately 500 mg of the meloxicam hydrogel was applied to the abdomen area and the subplantar area of the right hindpaw. Each group was treat-

ed once after carrageenan injection.

Statistics

All values are reported as a mean $\pm$ standard error of five determinations. The data were analyzed and the difference in the variables between the groups and the measuring periods was examined using one way analysis of variance (ANOVA) and Tukey post hoc tests. A p value < 0.05 was considered significant.

RESULTS

Primary experiment

Meloxicam hydrogel decreased paw swelling, indicating anti-hyperalgesia at 3, 6, and 9 h ($p < 0.001$), but no difference between the 1% and 2% meloxicam hydrogel groups (Fig. 1). Meloxicam hydrogel also improved the mechanical pain threshold after 3 ($p < 0.05$), 6, and 9 h ($p < 0.001$), but no difference between the 1% and 2% meloxicam hydrogel

Fig. 1. Effect of the application dose of meloxicam hydrogel on paw swelling. The error bar represents the standard deviation of the mean ($n=3$).

Fig. 2. Effect of the application dose of meloxicam hydrogel on paw mechanical pain threshold. The error bar represents the standard deviation of the mean ($n=3$).

groups (Fig. 2). Similarly, meloxicam hydrogel improved the thermal pain threshold after 3 ($p < 0.05$), 6, and 9 h ($p < 0.001$), with no difference between the 1% and 2% meloxicam hydrogel groups (Fig. 3). Therefore, 1% meloxicam gel was used at secondary experiment.

Secondary experiment

Meloxicam hydrogel delivered by phonophoresis decreased paw swelling after 3 and 6 h of application ($p < 0.01$) (Fig. 4), with no difference in paw or abdomen application. Meloxicam hydrogel phonophoresis also improved the mechanical pain threshold after 3 ($p < 0.05$), 6, and 9 h ($p < 0.001$) (Fig. 5). Meloxicam hydrogel phonophoresis decreased the thermal pain threshold after 3, 6, and 9 h ($p < 0.001$) (Fig. 6). Meloxicam hydrogel phonophoresis showed anti-inflammatory effects in the early inflammatory stage, with no differences between paw and abdomen application.

Fig. 3. Effect of the application dose of meloxicam hydrogel on paw thermal pain threshold. The error bar represents the standard deviation of the mean ($n=3$).

Fig. 4. Effect of the application site of meloxicam hydrogel on paw swelling. The error bar represents the standard deviation of the mean ($n=6$).

Fig. 5. Effect of the application site of meloxicam hydrogel on paw mechanical pain threshold. The error bar represents the standard deviation of the mean (n=6).

Fig. 6. Effect of the application site of meloxicam hydrogel on paw thermal pain threshold. The error bar represents the standard deviation of the mean (n=6).

DISCUSSION

We examined the possibility of using phonophoretic administration of meloxicam clinically by determining its anti-hyperalgesic effects in a rat carrageenan inflammation model. Carrageenan (1%) was injected into the plantar surface of the right hindpaw, and we measured the anti-edema and anti-hyperalgesic effects of meloxicam after phonophoretic transdermal delivery systemically or topically. Meloxicam activity was not different in the 1% and 2% groups (Fig. 1-3). Cui *et al.* (2008) reported no difference in 1, 3, or 5% meloxicam patches, and NSAID dose did not influence pain control in osteoarthritis patients (Yocum *et al.*, 2000).

NSAIDs do not change pain thresholds in acute inflammation (Sekiguchi *et al.*, 2008) suggesting that drug dose does not influence anti-hyperalgesic and anti-edema effects. Meloxicam hydrogel phonophoresis improved the mechanical and thermal pain threshold 3 and 6 h after car-

rageenan injection, as well as anti-hyperalgesic effects (Fig. 5, 6).

NSAIDs inhibit the synthesis of prostaglandins from arachidonic acid by inhibiting cyclo-oxygenase (COX) activity (Vane, 1971; Needleman *et al.*, 1986). COX-1 is involved in maintaining physiologic functions such as gastric protection and homeostasis, whereas COX-2 is involved in pathophysiologic process such as inflammation, pain, and fever (Gajraj, 2003). Meloxicam is a selective COX-2 inhibitor (Xin *et al.*, 2007), which mediates its anti-hyperalgesic effect (Abramson and Weissmann, 1989; Francischi *et al.*, 2002). Nociceptors are sensitized whenever the major COX-like prostaglandins are isolated from the inflammatory sites (Cesare and McNaughton, 1997; Chen *et al.*, 1999), which cause hyperalgesia (Ferreira, 1980). Meloxicam hydrogel produced similar or better effects than anti-inflammatory drugs using other administration routes (Dirig *et al.*, 1998; Jett *et al.*, 1999; Feltenstein *et al.*, 2004).

Meloxicam hydrogel phonophoresis reduced paw swelling from 3 to 6 h after carrageenan injection, demonstrating increased anti-inflammatory effects in the early inflammatory stage, with no difference in paw or abdomen application (Fig. 4). Phonophoresis application reduced paw swelling more than ultrasound and showed similar effects to systemic application. Phonophoresis application also improved mechanical and thermal pain thresholds better than the other groups (Fig. 5, 6). The inhibition of COX reduces the sensitization of peripheral afferents and therefore alleviates the hyperalgesia associated with tissue inflammation (Herrero *et al.*, 1997).

In many studies on phonophoresis, continuous ultrasound was used to enhance the delivery of anti-inflammatory drugs (Ciccone *et al.*, 1991; Kozanoglu *et al.*, 2003). Ultrasound waves are associated with nonthermal and mechanical effects such as vibration, pressure, and cavitation of cells as the waves pass through the tissue (Nyborg, 1985). The thermal effects can be reduced by using a pulsed mode of ultrasound, and these mechanical effects may facilitate drug permeation by vibrating the molecules in the tissue and drug media, altering the cell membrane and increasing cell permeability (Bommannan *et al.*, 1992).

NSAIDs are effective analgesics in situations of hyperalgesia related to tissue inflammation (Herrero *et al.*, 1997). The anti-inflammatory action of NSAIDs was caused by the suppression of the prostaglandin synthesis pathways by inhibiting the COX enzyme (Vane, 1971; Lim *et al.*, 2008). NSAIDs have similar anti-inflammatory effects in carrageenan or complete Freund adjuvant (CFA) induced inflammation (Hsieh, 2006). Iontophoresis of piroxicam (Penzes

et al., 2005) and diclofenac sodium (Gao *et al.*, 2002), phonophoresis of diclofenac sodium (Rosim *et al.*, 2005; Vlak, 1999), and phonophoresis of meloxicam gel (Choi *et al.*, 2006) could treat the musculoskeletal inflammatory disorders using common drugs by transdermal route. NSAIDs are used to treat inflammatory diseases of the musculoskeletal system such as arthritis, tendinitis and bursitis, where physical modalities are less effective.

Romsing *et al.* (2001) reported that pain control is similar 4 h after topical or injection. Topical application of NSAIDs has a systemic effect caused by systemic blood circulation (Carter *et al.*, 1997), and ketoprofen concentrations in tissue and blood were similar after phonophoretic application (Cagnie *et al.*, 2003). We found similar results after topical and systemic administration of meloxicam hydrogel by phonophoretic delivery, indicating that phonophoresis provides significant systemic delivery.

CONCLUSIONS

Phonophoretic transdermal delivery of a meloxicam hydrogel using pulsed ultrasound might be useful for treating acute inflammation via systemic or topical application.

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