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RESEARCH ARTICLE

The anti-inflammatory effect of diclofenac is considerably augmented by topical capsaicinoids-containing patch in carrageenan-induced paw oedema of rat

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Abstract Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most used drugs in musculoskeletal disorders, but their systemic adverse effects limit their therapeutic benefit in local inflammation. On the other hand, topical preparations of capsaicinoids are widely used for musculoskeletal disorders as a complementary therapy. In this study, the effects of both topical capsaicinoids-containing patch and local subcutaneous capsaicin application on the anti-inflammatory action of NSAID were examined. Carrageenan-induced paw oedema of rats was used as the inflammation model. The volume and weight of the paw oedema and plasma extravasation in the paw were determined after carrageenan injection. The systemic application of diclofenac (3 mg/kg), which is an NSAID, significantly decreased the volume and weight of the paw oedema. Topical capsaicinoids-containing patch application or local capsaicin injection (2, 10, 20 μg/paw) alone did not cause any effect on oedema volume and weight. However, the combination of diclofenac with topical capsaicinoids-containing patch significantly increased the effectiveness of diclofenac on inflammation. Evans blue content of the paws that represents plasma extravasation was decreased by capsaicinoids-containing patch with and without diclofenac and diclofenac combination with the lowest dose of capsaicin injection. The results of this study indicate that topical application of capsaicinoids-containing patch enhances the anti-inflammatory effect of diclofenac and its beneficial effect may not purely relate to its capsaicin content. In the treatment of local inflammatory disorders, the combination of NSAID with topical capsaicinoids-containing patch could increase the anti-inflammatory efficiency of drug without systemic side effects.

Keywords Capsaicin · Capsaicinoids · Diclofenac · Paw oedema · Topical patch

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are most commonly used to treat acute and chronic inflammations (Rao and Knaus 2008). Their therapeutic efficiency in systemic application could be insufficient in tissues with poor circulation. On the other hand, chronic and high-dose administrations of NSAIDs could cause serious side effects (Vonkeman and van de Laar 2010). Therefore, interventions to increase local efficiency are sometimes needed. Topical preparations of NSAIDs were approved, but there are questions about their clinical effectiveness. Individual differences to the response of drugs, skin side effects and uncorrelated levels of systemic and tissue NSAIDs concentrations are problems of topical therapy that still wait to be solved (Haroutiunian et al. 2010).

Capsaicin is the major pungent ingredient of hot peppers. Capsicum oleoresin is the alcoholic extract of peppers that contains capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin etc., all of which are called capsaicinoids (Szolcsányi 2004; Luo et al. 2011; Watanabe et al. 2011). Capsaicinoids have several pharmacological effects such as analgesia, anti-inflammation, anticancer, antioxidant and antiobesity (Luo et al.

2011). Capsaicin-containing patches and ointments are administered topically as an adjuvant analgesic in a variety of chronic painful diseases, including osteoarthritis, post-herpetic neuralgia, rheumatoid arthritis, post-mastectomy pain syndrome and diabetic neuropathy (Papoiu and Yosipovitch 2010; Anand and Bley 2011; Altman and Barthel 2011). Topical capsaicin application by acting on nociceptors of sensory neurons causes the secretion of various neuropeptides, which produces neurogenic inflammation characterized by vasodilatation and extravasation. After repeated, prolonged or high-dose application of capsaicin, neuronal desensitization occurs to various noxious stimuli, which is the mainstay of its analgesic activity (Steinhoff et al. 2003; Zegarska et al. 2006; Okajima and Harada 2006). Capsaicin-induced inflammation and anti-inflammatory effect of capsaicin were also reported (Wallengren and Möller 1986; Joe and Lokesh 1997; Kim et al. 2003; Manjunatha and Srinivasan 2006). It has been discussed that neuronal desensitization was responsible for its anti-inflammatory and analgesic effects as mentioned above.

The carrageenan-induced paw oedema of the rat is widely used to assess the anti-inflammatory effects of drugs (Vinegar et al. 1969; Garcia Leme et al. 1973; Morris 2003). Diclofenac is one of the most commonly prescribed NSAID with anti-inflammatory, analgesic and antipyretic properties (Gan 2010). However, the interaction between NSAIDs and capsaicinoids was not investigated in local inflammation. In the present study, the effects of topical capsaicinoids-containing patch and local capsaicin applications on the anti-inflammatory action of diclofenac were examined in the rat model of carrageenan-induced paw oedema.

Methods

Animal care and experimental protocol

All experiments were carried out in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The experimental procedure was approved by the Local Ethical Committee of Animal Care and Use of Ankara University. Female Wistar albino rats weighing 200–300 g were used in the experiments. The animals were housed in an air-conditioned room with 21 ± 1 °C ambient temperature under a 12-h light/dark cycle, where they had unlimited access to standard rat chow and water. Inflammation was induced by carrageenan in all of the rats and they were divided into ten groups randomly—(1) control: no therapy was given or only patches with no drug content were applied; (2) diclofenac: diclofenac (3 mg/kg) was injected intramuscularly; (3) patch: capsaicinoids-containing patch was applied; (4) patch + diclofenac: capsaicinoids-containing patch was applied and diclofenac was injected intramuscularly; (5) capsaicin 2 μg: capsaicin 2 μg was injected into the paw; (6) capsaicin 2 μg + diclofenac: capsaicin 2 μg was injected into the paw and diclofenac was injected intramuscularly; (7) capsaicin 10 μg: capsaicin 10 μg was injected into the paw; (8) capsaicin 10 μg + diclofenac: capsaicin 10 μg was injected into the paw and diclofenac was injected intramuscularly; (9) capsaicin 20 μg: capsaicin 20 μg was injected into the paw; (10) capsaicin 20 μg + diclofenac: capsaicin 20 μg was injected into the paw and diclofenac was injected intramuscularly.

Inflammation was obtained using the carrageenan-induced paw oedema model of rat. Carrageenan was suspended in saline (%1 w/v) and injected intraplantarly (1 mg/0.1 ml/paw) in the right hind paws of all the rats. The same volume of saline was injected into the left hind paws of all the animals. The carrageenan injection time was regarded as zero. 90 min after carrageenan injection, diclofenac (3 mg/kg) was injected into the left thigh of the rats intramuscularly.

Capsicum oleoresin-containing patches (approximately 260 μg capsicum oleoresin and 20 μg capsaicin in 1.2 cm diametric circular patch) were applied to the right hind paws of the rats in the patch and patch + diclofenac groups after carrageenan injection. Patches with no drug content were applied to the left hind paws of the rats in the patch and patch + diclofenac groups and to the right and left hind paws of the rats in the diclofenac group. In a different day of the study, control animals of each day were included in the experiment for the assessment of carrageenan efficiency. When the patch group was examined, patches with no drug were applied to the control animals. At the end of the study, the values of the control animals in different working days were not different and pooled together.

Capsaicin was dissolved in a solution composed of 80 % saline, 10 % ethanol and % 10 tween 80 and injected subcutaneously (2, 10 or 20 μg/20 μl/paw) in the right hind paws of the rats. The same volume of the vehicle solution was injected into the left hind paw. Capsaicin or its vehicle was administered immediately after carrageenan injection.

The volumes of both hind paws were measured using a plethysmometer (Ugo Basile 7140, USA) in all groups at the 0, 180, 270 and 360th min. If a patch was applied, it was removed before the measurement and reapplied after drying the paw. The volume difference between right and left paws was regarded as paw oedema volume.

Plasma extravasation was measured using Evan's blue method as described with modifications (Saria 1984). Rats were anaesthetized with an intraperitoneal injection of ketamine (80 mg/kg) + xylazine (10 mg/kg). Evans blue dye at a dose of 60 mg/kg was administered by intracardiac injection at the 345th min. After the paw volume

measurements at the 360th min, the left carotid artery was cut and the rats were killed by exsanguination, then both hind paws were cut at the wrist joint. The paws were weighed immediately to determine wet weights. The paws were dried by incubation at 56 °C for 48 h and weighed again determining the dry weights. The difference between the wet weight and dry weight of an individual paw was regarded as the fluid mass of that paw and the difference of the fluid masses of the right and left paw was regarded as the paw oedema weight. Paws were incubated in formamide at 56 °C for 48 h, to extract the extravasated Evan's blue. The absorbance of the extracted dye was determined spectrophotometrically at 620 nm, and the concentration was calculated using a standard absorbance curve obtained from known concentrations of Evans blue in formamide. Results are expressed as μg dye/mg dry tissue of the paw.

Chemicals

Carrageenan (λ type 1) and capsaicin (product number: 21750 LOT: 1437650) were purchased from Sigma Aldrich (St. Louis, MO, USA). Capasicum oleoresin-containing patches were obtained from Sanli Drug Industries (Turkey), diclofenac (Dikloron) was from Deva Drug Company (Turkey) and formamide was from Flucka, Switzerland.

Statistical analysis

Values are expressed as mean ± SEM. Statistical analysis was performed using SigmaPlot (Systat Software Inc., San Jose, CA, USA), version 11 for Windows. The time course of the paw oedema was compared by using repeated measures of two-way ANOVA and, if a statistically significant difference was found, further comparisons were made using the Holm–Sidak test. The paw oedema weight and Evans blue content were compared between groups using Student's *t* test. Values were considered significantly different when $p < 0.05$.

Results

In the beginning of the experiments, rats' weight was measured and there were no differences between groups. Immediately after the carrageenan injection, the paw volumes of rats were similar in all groups at time point zero. The time courses of the paw oedema of all the groups are shown in Fig. 1.

Diclofenac alone significantly decreased the paw oedema volume at the 180th min (0.59 ± 0.04 ml in the control and 0.39 ± 0.05 ml in diclofenac groups) and 270th min (0.67 ± 0.05 ml in control and 0.35 ± 0.06 ml in the diclofenac groups) ($p < 0.05$), but the oedema formation in the

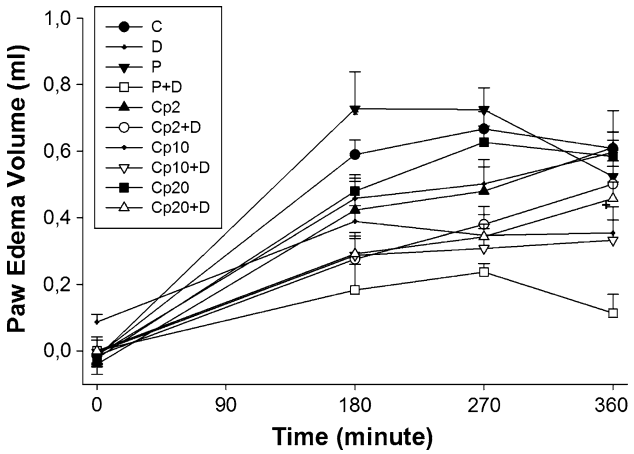

Fig. 1 Effects of diclofenac, patch and capsaicin on the carrageenan-induced paw oedema volume of rats. Patch application potentiated diclofenac-induced decrease in paw oedema volume. *C* control, *D* diclofenac, *P* patch, *Cp2* capsaicin 2 μg, *Cp10* capsaicin 10 μg, *Cp20* capsaicin 20 μg. Values are expressed as mean ± SEM ($n = 10$–17)

360th min was not affected by diclofenac (Fig. 2). On the other hand, diclofenac with capsaicinoids-containing patch significantly decreased the oedema volume greater than the control and diclofenac groups at the 180th min (0.18 ± 0.08 ml in patch + diclofenac group) and at the 360th min (0.61 ± 0.05 ml in control, 0.35 ± 0.08 ml in diclofenac and 0.11 ± 0.06 ml in patch + diclofenac groups) ($p < 0.05$) (Fig. 2). It was hypothesized that capsaicin would be responsible for this effect, because capsaicin is the main active substance in capsaicinoids-containing patch. The combination of diclofenac with different doses of capsaicin also caused inhibition of oedema formation, but it was not different from diclofenac alone (Fig. 2). Patch or three doses of capsaicin application without diclofenac did not alter oedema formation at all time points of the experiments (Fig. 2).

The application of diclofenac caused a significant decrease in paw oedema weight (413.24 ± 36.50 mg in control and 242.00 ± 37.36 mg in diclofenac groups) ($p < 0.05$) (Fig. 3). The combination of diclofenac with patch, capsaicin 2 and 10 μg also significantly reduced oedema weight, but they were not different from diclofenac alone group. The oedema weights of other groups were not different from the control (Fig. 3).

Evans blue molecule binds to albumin with a very high affinity; therefore, the concentration of Evans blue is highly correlated with tissue albumin content that is elevated by vascular permeability increase. Thus, the Evans blue concentration of tissue is an index of plasma extravasation that represented the last 15 min of permeation in the present study. Patch and diclofenac with patch and capsaicin (2 μg) applications significantly decreased tissue Evans blue content of the inflamed right paws (33.08 ± 2.620 μg/mg

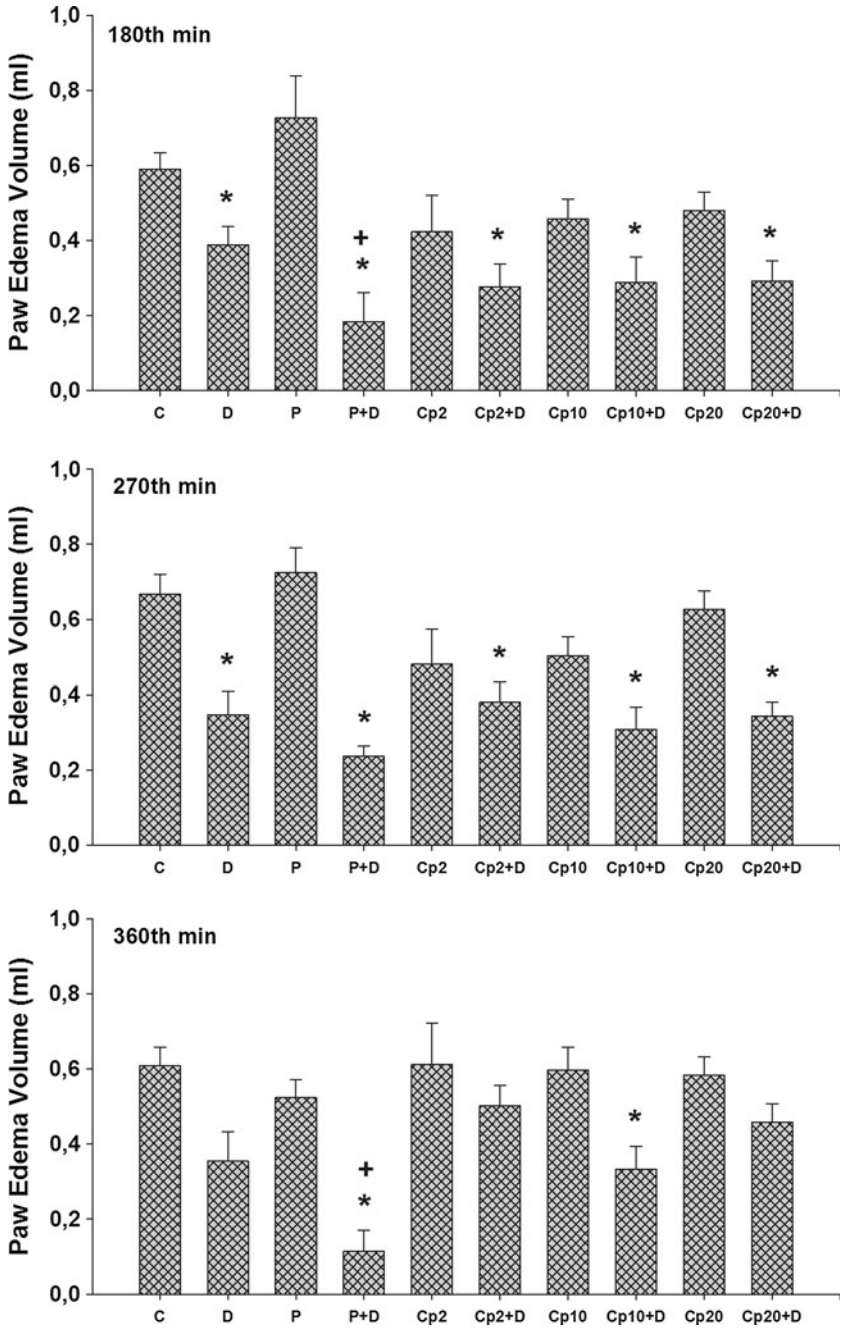

Fig. 2 Effects of diclofenac, patch and capsaicin on the carrageenan-induced paw oedema volume of rats at different time points. While diclofenac alone significantly prevented paw oedema volume in the 180th and 270th min, combination of diclofenac with patch significantly inhibited oedema formation at all time points ($p < 0.05$). *C* control, *D* diclofenac, *P* patch, *Cp2* capsaicin 2 μg, *Cp10* capsaicin 10 μg, *Cp20* capsaicin 20 μg. Differences from control (*) and diclofenac (+) groups. Values are expressed as mean ± SEM ($n = 10$–17)

in control, 22.64 ± 1.91 μg/mg in patch, 17.10 ± 1.89 μg/mg in patch + diclofenac and 25.39 ± 0.90 μg/mg in capsaicin 2 μg + diclofenac groups) ($p < 0.05$) (Fig. 4). Evans blue content of the other groups was not different from that of the control (Fig. 4).

Discussion

The results of this study show that the anti-inflammatory action of diclofenac in the carageenan-induced paw oedema of rats is significantly augmented by topical applications of capsaicinoids-containing patch, but not by local capsaicin injection.

Inflammation is fundamentally a protective response designed to relieve and repair tissues from damage, such as that by microbes, toxins and trauma. The mechanism designed to destroy foreign invaders and necrotic tissues has an intrinsic ability to injure normal tissues. A variety of mediators are released during the inflammation including prostaglandins, histamine, serotonin, kinin peptides, leukotrienes, platelet-activating factor, reactive oxygen species, nitric oxide, chemokines and cytokines (Cotran et al. 2009). Inappropriately directed or controlled

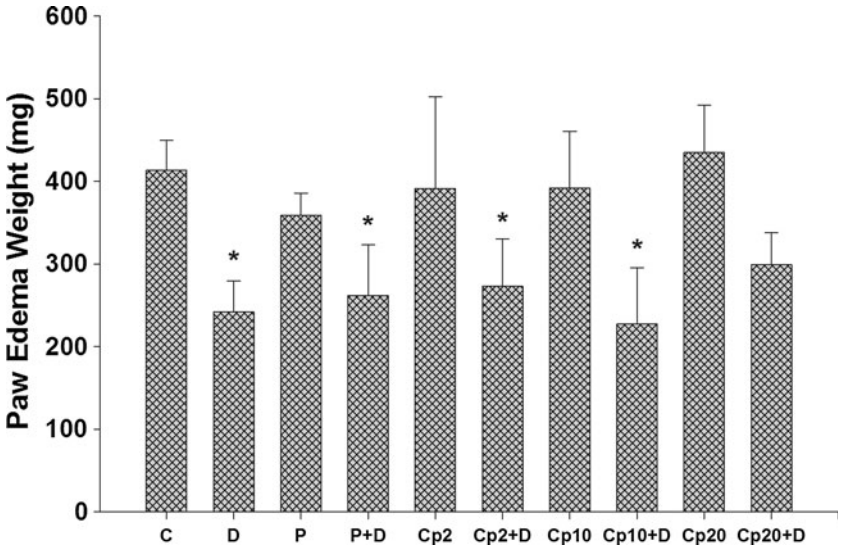

Fig. 3 Effects of diclofenac, patch and capsaicin on the carrageenan-induced paw oedema weight of rats. Diclofenac alone and combination of patch or capsaicin significantly prevented paw oedema weight ($p < 0.05$). *C* control, *D* diclofenac, *P* patch, *Cp2* capsaicin 2 μg, *Cp10* capsaicin 10 μg, *Cp20* capsaicin 20 μg. Differences from control (*) group. Values are expressed as mean ± SEM ($n = 9$–17)

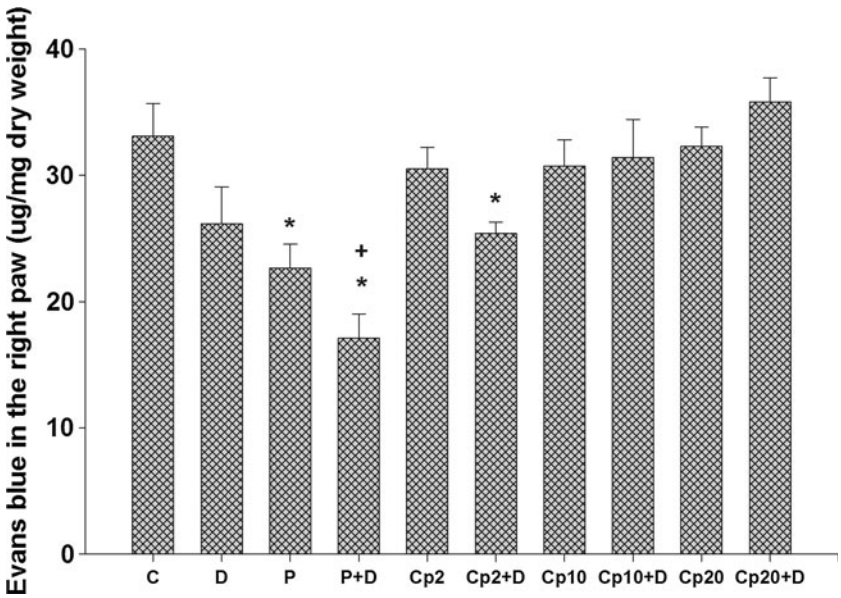

Fig. 4 Effects of diclofenac, patch and capsaicin on the tissue Evans blue content of the right paw. Although diclofenac has no effect on tissue Evans blue content, application of patch, patch with diclofenac and capsaicin with diclofenac significantly decreased dye concentration in paws ($p < 0.05$). *C* control, *D* diclofenac, *P* patch, *Cp2* capsaicin 2 μg, *Cp10* capsaicin 10 μg, *Cp20* capsaicin 20 μg. Differences from control (*) and diclofenac (+) groups. Values are expressed as mean ± SEM ($n = 6$–16)

inflammation causes disease and the therapy usually targets the inhibition of synthesis or effects of substances that mediate inflammatory reactions. NSAIDs are the most commonly used drugs (Rao and Knaus 2008). NSAIDs decrease prostanoid production due to inhibition of cyclo-oxygenase enzymes and effectively influence the inflammation (Rao and Knaus 2008; Ricciotti and Fitz-Gerald 2011). However, serious side effects limited their therapeutic effectiveness (Vonkeman and van de Laar 2010). Especially in some musculoskeletal disorders, because of poor circulation in inflamed tissue, insufficiency of therapeutic benefit was obscure and high doses and long periods of application were needed. The topical application of these drugs is one of the methods used for avoiding side effects. On the other hand, it has been reported that the topical application of NSAIDs had several therapeutic problems such as uncorrelated tissue and systemic concentrations, individual differences in efficacy and side effects on the skin (Haroutiunian et al. 2010; Altman and Barthel 2011). Because of all these problems, treatment of local musculoskeletal inflammation needs further approach.

Carrageenan-induced pathology in tissues is documented as an inflammation and widely used as an experimental model to study the pathogenesis and therapeutic approach of inflammatory diseases (Vinegar et al. 1969; Garcia Leme et al. 1973; Manjunatha and Srinivasan 2006). Intraplantar injection of carrageenan into the rat paw causes inflammatory oedema. Diclofenac is frequently used as the reference drug for evaluating the anti-inflammatory effect of other treatment modalities in this animal model (Gan 2010). After the application of diclofenac, the

paw oedema volume decreased at the 180th and 270th min in our study. However, the oedema volume and the tissue Evans blue content were not significantly altered by diclofenac at the 360th min, and decreased oedema weight was recorded.

Hot peppers contain several capsaicinoids including capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Szolcsányi 2004; Luo et al. 2011; Watanabe et al. 2011). Capsaicinoids-containing patches without a prescription are used for treatment of musculoskeletal pain as a complementary therapeutic. Although NSAIDs and patches have been used separately, the interaction of NSAIDs with capsaicinoids-containing patches was not examined. The present study firstly showed that the action of diclofenac on inflammation was potentiated by topical capsaicinoids-containing patch in rat paw oedema models of inflammation.

Capsaicin is the major pungent ingredient in capsaicinoids (Szolcsányi 2004; Luo et al. 2011; Watanabe et al. 2011). It has been described that capsaicin has a variety of pharmacological effects such as analgesia, anticancer, antioxidant and antiobesity (Luo et al. 2011). Topical capsaicin application was approved as an analgesic in a variety of chronic painful diseases, including osteoarthritis, post-herpetic neuralgia, rheumatoid arthritis, post-mastectomy pain syndrome and diabetic neuropathy (Papoiu and Yosipovitch 2010; Anand and Bley 2011; Altman and Barthel 2011). Topical capsaicin application in the beginning causes pain, but repeated, prolonged or high-dose applications induce neuronal desensitization and prevent signal transmission, thus ending pain (Steinhoff et al. 2003; Zegarska et al. 2006; Okajima and Harada 2006). In addition, the anti-inflammatory effect of capsaicin and capsaicin-triggered inflammation were also reported (Wallengren and Möller 1986; Joe and Lokesh 1997; Kim et al. 2003; Manjunatha and Srinivasan 2006). In our study, the capsaicin content of the applied patches was approximately 20 μg and there were no data on the bioavailability of the topical capsaicin in rat skin. Thus, this and smaller doses of capsaicin were selected. Local subcutaneous injections of capsaicin without diclofenac did not change oedema volume, weight and plasma extravasation of the rat paw in the present study. In addition, a combination of diclofenac with a local injection of capsaicin did not show a different effect from diclofenac alone on carrageenan-induced inflammation in the current study. Different effects of capsaicin on inflammation were described in previous studies as mentioned above, but experimental protocols and models were completely different from our study. Because of using different doses, timing, applications and models, it is difficult to compare the data and understand the nature of capsaicin action. Further studies are needed for clarifying the dose and time-dependent effects of capsaicin on inflammation in different experimental models.

Capsaicinoids-containing patch comprise various active carotenoids and phenolic compounds such as vitamins A and C, lutein, lycopene, β-cryptoxanthin, β-carotene, quercetin, catechin, rutin, vanillin and gallic, protocatechic, ferulic, coumaric, sinapinic and caffeic acids (Wilbur 2007; Troconis-Torres et al. 2012). Most of these substances exhibit antioxidant properties which may cause anti-inflammatory action (Guo et al. 2009; Zhang et al. 2011). Thus, it is posited that the beneficial effect of topical capsaicinoids-containing patch on anti-inflammatory actions of NSAID may depend on another active substance(s) in red pepper. This hypothesis is supported by the study that reported the anti-inflammatory action of pepper juice (Spiller et al. 2008). However, further studies to elucidate localization (local or systemic) of interaction and molecular mechanism(s) behind the synergy of the capsaicinoids-containing patch and diclofenac are needed.

All these results indicate that capsaicinoids-containing patch augments anti-inflammatory action of diclofenac in carrageenan-induced rat paw oedema and its beneficial effect might be related to other active substance(s) of red peppers in the patch. Determining the active substance(s) and molecular mechanism of interaction may lead to the development of novel strategies for the effective treatment of localized inflammation with NSAIDs without side effects.

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References

Altman RD, Barthel HR (2011) Topical therapies for osteoarthritis. Drugs 71(10):1259–1279

Anand P, Bley K (2011) Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. Br J Anaesth 107(4):490–502

Cotran R, Kumar V, Collins T (2009) Acute and chronic inflammation. In: Kumar V, Abbas AK, Fausto N, Aster J (eds) Pathologic basis of disease, 6th edn. Saunders, Philadelphia, pp 43–57

Gan TJ (2010) Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin 26(7):1715–1731

Garcia Leme J, Hamamura L, Leite MP, Rocha e Silva M (1973) Pharmacological analysis of the acute inflammatory process induced in the rat's paw by local injection of carrageenin and by heating. Br J Pharmacol 48(1):88–96

Guo W, Kong E, Meydani M (2009) Dietary polyphenols, inflammation, and cancer. Nutr Cancer 61(6):807–810

Haroutiunian S, Drennan DA, Lipman AG (2010) Topical NSAID therapy for musculoskeletal pain. Pain Med 11(4):535–549

Joe B, Lokesh BR (1997) Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. Lipids 32(11):1173–1180

Kim CS, Kawada T, Kim BS, Han IS, Choe SY, Kurata T, Yu R (2003) Capsaicin exhibits anti-inflammatory property by inhibiting IkB-a degradation in LPS-stimulated peritoneal macrophages. Cell Signal 15(3):299–306

Luo XJ, Peng J, Li YJ (2011) Recent advances in the study on capsaicinoids and capsinoids. Eur J Pharmacol 650(1):1–7

Manjunatha H, Srinivasan K (2006) Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. FEBS J 273(19): 4528–4537

Morris CJ (2003) Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol 225:115–121

Okajima K, Harada N (2006) Regulation of inflammatory responses by sensory neurons: molecular mechanism(s) and possible therapeutic applications. Curr Med Chem 13(19):2241–2251

Papoiu AD, Yosipovitch G (2010) Topical capsaicin. The fire of a 'hot' medicine is reignited. Expert Opin Pharmacother 11(8):1359–1371

Rao P, Knaus EE (2008) Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J Pharm Pharm Sci 11(2):81s–110s

Ricciotti E, FitzGerald GA (2011) Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31(5):986–1000

Saria A (1984) Substance P in sensory nerve fibres contributes to the development of oedema in the rat hind paw after thermal injury. Br J Pharmacol 82(1):217–222

Spiller F, Alves MK, Vieira SM, Carvalho TA, Leite CE, Lunardelli A, Poloni JA, Cunha FQ, de Oliveira JR (2008) Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan- and antigen-induced inflammation. J Pharm Pharmacol 60(4):473–478

Steinhoff M, Ständer S, Seeliger S, Ansel JC, Schmelz M, Luger T (2003) Modern aspects of cutaneous neurogenic inflammation. Arch Dermatol 139(11):1479–1488

Szolcsányi J (2004) Forty years in capsaicin research for sensory pharmacology and physiology. Neuropeptides 38(6):377–384

Troconis-Torres IG, Rojas-López M, Hernández-Rodríguez C, Villa-Tanaca L, Maldonado-Mendoza IE, Dorantes-Álvarez L, Tellez-Medina D, Jaramillo-Flores ME (2012) Biochemical and molecular analysis of some commercial samples of chilli peppers from Mexico. J Biomed Biotechnol 2012:873090

Vinegar R, Schreiber W, Hugo R (1969) Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther 166(1):96–103

Vonkeman HE, van de Laar MA (2010) Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. Semin Arthritis Rheum 39(4):294–312

Wallengren J, Möller H (1986) The effect of capsaicin on some experimental inflammations in human skin. Acta Derm Venereol 66(5):375–380

Watanabe T, Ohnuki K, Kobata K (2011) Studies on the metabolism and toxicology of emerging capsinoids. Expert Opin Drug Metab Toxicol 7(5):533–542

Wilbur J (2007) Final report on the safety assessment of *Capsicum annuum* extract, *Capsicum annuum* fruit extract, *Capsicum annuum* resin, *Capsicum annuum* fruit powder, *Capsicum frutescens* fruit, *Capsicum frutescens* fruit extract, *Capsicum frutescens* resin, and capsaicin. Int J Toxicol 26(Suppl 1):3–106

Zegarska B, Lelińska A, Tyrakowski T (2006) Clinical and experimental aspects of cutaneous neurogenic inflammation. Pharmacol Rep 58(1):13–21

Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, Bartlett J, Shanmugam K, Münch G, Wu MJ (2011) Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem 59(23):12361–12367