Evaluation of Topical Gels Containing Non-Steroidal Anti-Inflammatory Drugs on Inflammation and Hyperalgesia in rats

S Padi, M Gupta, J Kehal, A Aggarwal, N Kumar, R Marwaha

Citation

Abstract
The systemic use of non-steroidal anti-inflammatory drugs (NSAIDs) which act by inhibiting cyclooxygenase (COX) is severely hampered by gastric and peptic ulcers. The topical delivery of NSAIDs has the advantages of avoiding gastric and peptic ulcers and delivering the drug to the inflammation site. There are no studies that compared the pharmacological profile of gel formulations containing different NSAIDs. Therefore, attempt has been made to study the anti-inflammatory and antihyperalgesic effects of NIZER gel (nimesulide, a preferential COX-2 inhibitor, 1 mg per 100 mg gel) and VOVERAN Emulgel (diclofenac sodium, a nonselective COX (COX-1/2) inhibitor, 1 mg per 100 mg gel) in carrageenan-induced inflammation and hyperalgesia in rats. A 100 mg of NIZER gel or VOVERAN Emulgel when applied topically 30 min before inflammogen administration showed marked anti-inflammatory and antihyperalgesic effects against carrageenan-induced inflammation and hyperalgesia in rats with more significant effect was observed with NIZER gel. The results indicate that gels containing a preferential COX-2 inhibitor are better than a non-selective COX-1/2 inhibitor in alleviating inflammation and hyperalgesia.

INTRODUCTION
Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of fever, acute and chronic arthritic conditions. They act by inhibiting cyclooxygenase (COX) thereby reducing the release of prostaglandins (PGs), well known inflammatory and nociceptive mediators (Dirig et al., 1998; Padi and Kulkarni, 2004; Padi et al., 2004). PGs are gastroprotective in that they enhance the synthesis mucosa, bicarbonate secretion and reduce the gastric acid secretion. However, their systemic use is often limited because of severe upper gastrointestinal ulcers and other side effects (Watson et al., 2000; Padi et al., 2004; Ray et al., 2007; Süleyman et al., 2007). Thus, pharmaceutical dosage forms that deliver drugs to the inflammation site at a sustained, concentrated level over an extended period of time without altering pharmacodynamic activities have the advantages of avoiding the systemic side effects.

In the recent time, transdermal drug delivery systems have gained much importance for local but sustained action of many therapeutic agents including anesthetics and analgesics. Most importantly, NSAIDs are incorporated in topical formulations that are designed to deliver drugs at appropriate rates to maintain minimum plasma drug levels for therapeutic efficacy by using skin as the port of entry of drugs (Bhaskaran et al., 2000; Brown et al., 2006; Gupta et al., 2008). One side the topical applications of the drug offer the potential advantages of delivering the drug directly to the site of action and delivering the drug for extended period of time at the inflammation site that mainly acts at the joint and the related regions. On the other hand, topical delivery system increases the contact time and mean resident time of drug at the applied site leading to an increase in local drug concentration while the pharmacological activity of gel formulations may not change as rapidly as the solution form (Brown et al., 2006; Kumar and Philip, 2007).

It is well known that transdermal gels are more popular among all topical preparations due to ease of application and better percutaneous absorption than other semisolid dosage forms. The effect of various excipients added in varied concentrations on transcutaneous drug permeation has been explored where the permeation rate of a topical agent may be influenced by drug-vehicle, drug-skin and vehicle-skin interaction (Giannakou et al., 1995; Wu et al., 2000; Omidian et al., 2007). Numerous studies exist in the
literature that reports the amount of drug released and its time and dose-dependent release pattern in in vitro and ex vivo models. However, few studies on topical gel formulations reported the therapeutic effects of drugs in a variety of experimental inflammation and inflammatory pain conditions (Gupta et al., 2002; Huang et al., 2007; Gupta et al., 2008). Further, reasonably few attempts have been made to identify relative therapeutic efficacy of such formulations.

Therefore, the present was designed to evaluate the relative pharmacodynamic profile of commercially available gel formulations containing a NSAID, particularly diclofenac, a non-selective COX-1/2 inhibitor or nimesulide, a preferential selective COX-2 inhibitor along with different polymers and permeation enhancers in an in vivo experimental model.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Wistar rats (180 – 200 g) (Central Animal House of I.S.F. College of Pharmacy, Moga, India) of either sex were used in the experiments. All procedures involving the use of animals were approved by the Institutional Animal Ethics Committee and carried out in accordance with the guidelines of the Indian National Science Academy. All the experiments were performed between 9:00 and 16:00 h. All the animals were kept under standard conditions of light and dark cycle with food and water ad libitum in groups of 3 animals in plastic cages with soft bedding. The animals were allowed to acclimatize for one week before the experiments.

DRUGS AND TREATMENT SCHEDULE

Nizer Gel (1% w/w nimesulide, 5% w/w menthol, 10% w/w methyl salicylate in water soluble base, USV Ltd., India), VOVERAN Emulgel (1.16% w/w diclofenac diethylammonium equivalent to diclofenac sodium in water soluble base, Novartis, India), carrageenan λ (type IV) (Sigma-Aldrich, India) were used in this study. In all the experiments, 100 mg gel formulation was gently applied to the left hind paw of rats 30 min before carrageenan injection.

CARRAGEENAN-INDUCED PAW EDEMA

In order to measure paw volume, animals were marked with a permanent marker at the ankle of their left hind paws to define the area of the paw to be monitored. Paw edema was induced by injecting 100 µl of a 1% solution of λ-carrageenan in normal saline into the plantar surface of the left hind paw of the rats (Padi et al., 2004). The paw volumes were measured using a plethysmometer at 0 (before), 0.5, 1, 1.5, 2, 3, and 4 h after carrageenan administration, and the change in paw volume (V₄₉ – V₀₉) 4 h after carrageenan administration in control and drug-treated animals was calculated. The % increase in edema at each time point for both the gel formulation was also calculated in comparison to control group. The anti-inflammatory activity is expressed as percent inhibition of paw edema and was calculated by taking the values in the control group as 0% inhibition.

CARRAGEENAN-INDUCED THERMAL HYPERALGESIA

Hyperalgesia was induced by injecting 100 µl of a 1% solution of λ-carrageenan (Sigma, USA) in normal saline into the plantar surface of the left hind paw of rats. In all animals, thermal hyperalgesia was measured using the procedure described by Padi et al. (2004). The mean paw withdrawal latency of the left paw when dipped in water bath maintained at 47 ± 0.5 °C was measured 0 (before), 0.5, 1, 1.5, 2, 2.5, 3, and 4 h after carrageenan injection. The baseline latency of paw withdrawal from thermal source was established three times, 5 min apart, and averaged. A cut-off time of 15 sec was imposed to avoid injury to the paw. The mean paw withdrawal latency (L₄₉) 4 h after carrageenan administration in control and drug-treated animals was measured and the change in the paw withdrawal latency (L₀₉ – L₄₉) was calculated as a measure of hyperalgesia. Antihyperalgesic activity is expressed as percent inhibition of hyperalgesia and was calculated by taking the values in the control group as 0% inhibition.

STATISTICAL ANALYSIS

All the values are expressed as mean ± S.E.M. and the date were analyzed by one-way analysis of variance with Tukey's test for multiple comparisons. The mean values between two groups were analyzed by unpaired t-test. In all the analyses P < 0.05 was considered as statistically significant.

RESULTS

CARRAGEENAN-INDUCED PAW EDEMA

Subcutaneous administration of carrageenan into the hind paw produced significant edema, with a maximum response being observed 4 h after injection. NIZER gel (1 mg nimesulide, 5 mg menthol, 10 mg methyl salicylate per 100 mg gel) or VOVERAN Emulgel (1 mg diclofenac sodium per 100 mg gel), when applied topically 30 min before carrageenan administration, inhibited paw edema in a time-
dependent manner (Fig. 1) with an anti-inflammatory effect (% edema inhibition) of 66.19 ± 6.59 and 43.53 ± 4.38, respectively was achieved at 3 h (Fig. 2). The anti-inflammatory activity of NIZER gel was better and significantly different from that of VOVERAN Emulgel at 3 h after carrageenan administration (Fig. 2).

**Figure 1**
Figure 1: Time-dependent inhibitory effect of topically applied 100 mg NIZER gel or VOVERAN Emulgel against carrageenan-induced paw edema in rats. Carrageenan (100 μg per paw) was injected subplantarly 30 min after applying gel formulation. Values are mean ± S.E.M. < 0.05 as compared to carrageenan control. < 0.05 as compared to ipsilateral VOVERAN Emulgel applied paw.

**Figure 2**
Figure 2: Anti-inflammatory effect of topically applied 100 mg NIZER gel or VOVERAN Emulgel against carrageenan-induced paw edema in rats. Carrageenan (100 μg per paw) was injected subplantarly 30 min after applying gel formulation. The percent inhibition of edema was calculated 3 h after carrageenan administration by taking the values in the control group as 0% inhibition. Values are mean ± S.E.M. <0.05 as compared to ipsilateral VOVERAN Emulgel applied paw.

**HYPERALGESIA**
Carrageenan administration into the hind paw produced a significant inflammation associated with hyperalgesia as shown by decreased paw withdrawal latency in response to a thermal stimulus at 47 ± 0.5 °C for 4 h after carrageenan injection. Both NIZER gel (1 mg nimesulide, 5 mg menthol, 10 mg methyl salicylate per 100 mg gel) or VOVERAN Emulgel (1 mg diclofenac sodium per 100 mg gel) showed time-dependent inhibition of carrageenan-induced thermal hyperalgesia (Fig. 3) with almost 77.77 ± 6.58 and 41.38 ± 3.93 % inhibition, respectively was achieved at 3 h (Fig. 4). The antihyperalgesic effect of NIZER gel was comparatively better and significantly different from VOVERAN Emulgel at 3 h after carrageenan injection (Fig. 4).

**Figure 3**
Figure 3: Time-dependent inhibitory effect of topically applied 100 mg NIZER gel or VOVERAN Emulgel against carrageenan-induced thermal hyperalgesia in rats. Carrageenan (100 μg per paw) was injected subplantarly 30 min after applying gel formulation. Values are mean ± S.E.M. <0.05 as compared to contralateral (non-carrageenan-injected) paw, <0.05 as compared to ipsilateral (carrageenan-injected) paw. <0.05 as compared to VOVERAN Emulgel.
Figure 4: Antihyperalgesic effect of topically applied NIZER gel or VOVERAN Emulgel against carrageenan-induced thermal hyperalgesia in rats. Carrageenan (100 µg per paw) was injected subplantarly 30 min after applying gel formulation. The percent inhibition of hyperalgesia was calculated 3 h after carrageenan administration by taking the values in the control group as 0% inhibition. Values are mean ± S.E.M. <0.05 as compared to VOVERAN Emulgel.

DISCUSSION

The present study systematically investigated the time-dependent pharmacodynamic profile of commercially available topical analgesic and anti-inflammatory gel formulations containing a NSAID in a water soluble base. It has been previously reported that topical application of NSAID containing formulations in animals can markedly attenuate inflammation, pain and related behaviors (Gupta et al., 2002; Huang et al., 2007; Gupta et al., 2008). We have extended this observation to show that gels containing diclofenac, a nonselective COX (COX-1/2) inhibitor (Watson et al., 2000) or nimesulide, which is a preferential COX-2 inhibitor (Rainsford, 2006), can also attenuate the inflammation and associated pain in experimental model of inflammation.

PGs play an important role in promoting the signs and symptoms of inflammation and they sensitize terminal afferent C-fibers in the periphery and enhance the response of C-fibers to algic stimuli resulting in hyperalgesia (Vanegas and Schaible, 2001; Padi et al., 2004; Svensson ands Yaksh, 2002). One of the defining features of inflammatory pain is a pronounced hypersensitivity to noxious mechanical and thermal stimulation of the skin (Chen et al., 1999; Schmelz et al., 2003; Satyanarayana et al., 2004). Thus, carrageenan-induced paw edema is the most commonly used test for studying anti-inflammatory activity and hyperalgesia in animals. In the present study, prophylactic administration of VOVERAN emulgel or NIZER gel resulted in the time-dependent inhibition of both carrageenan-induced inflammation and hyperalgesia during the experimental period. Consistent with previous studies where maximum inflammation and hyperalgesia developed 2 – 4 h after carrageenan administration (Beiche et al., 1996; Dirig et al., 1998; Padi et al., 2004), in the present study, the peak anti-inflammatory and antihyperalgesic effect was observed 3 h after carrageenan administration. Further, approximately 65% inhibition of inflammation and almost 75% inhibition of hyperalgesia at 3 h following inflammogen injection were observed with NIZER gel. In contrast, the same dose of VOVERAN emulgel gel caused about 40% inhibition of inflammation and thermal hyperalgesia.

The present results are consistent with previous reports in which systemically as well as spinal administered nonselective COX or selective COX-2 inhibitors were effective (Dirig et al., 1998; Yaksh et al., 2001; Padi et al., 2004). Further, it has been reported that COX-2 mRNA and protein in spinal cord increased while spinal levels of COX-1 mRNA were not altered 3 – 6 h after carrageenan injection in the hind paw (Beiche et al., 1996; Dirig et al., 1998; Prochazkova et al., 2006). It is well known that COX-2 is inducible whereas COX-1 is constitutive, it could be expected that the initial inflammation mediated by PGs derived from activation of COX-1 rather than from COX-2 in carrageenan-induced inflammation. It has been reported that increased COX-2 immunoreactivity in the epidermis, skeletal muscle and inflammatory cells of rats experiencing hyperalgesia was observed, however, in paw edema, only the epidermis showed positive COX-2 immunoreactivity (Nantel et al., 1999). In addition, carrageenan injection induced expression of COX-2-like immunoreactivity in vascular endothelial cells throughout the CNS which became evident by 3 h and was most prominent at 6 h after inflammogen injection. This COX-2 induction was associated with an elevation of PGE2 in the cerebrospinal fluid (Ibuki et al., 2003). Furthermore, such intrathecally evoked release of spinal PGE2 was diminished by systemic delivery of nonspecific COX and COX-2-selective inhibitors, but not a COX-1-selective inhibitor. Thus, constitutive spinal COX-2, but not COX-1, is also an important contributor to the acute antihyperalgesic effects of spinal as well as systemic COX-2 inhibitors (Dirig et al., 1998; Yaksh et al., 2001). A recent study reported that inhibition of COX-2 by intraplantar (paw) injection of nimesulide increases the levels of
endocannabinoid related molecules and attenuated both hyperalgesia and hind paw edema via peroxisome proliferator-activated receptor-alpha in a model of inflammatory pain suggesting that COX-2 play a role in the metabolism of endocannabinoids and related molecules (Jhaveri et al., 2008).

It has been reported that intraplantar injection of soluble peroxynitrite itself induces inflammatory hyperalgesia, which activates the transcription factor nuclear factor (NF)-κB in paw tissues and enhances expression of the inducible COX-2 but not the constitutive COX-1 enzyme. Importantly, in that study, peroxynitrite-mediated hyperalgesia was blocked by indomethacin, a nonselective COX-1/2 inhibitor or NS398, a selective COX-2 inhibitor, as well as by an anti-PGE2 antibody. In addition, the peroxynitrite decomposition catalyst synergized with both indomethacin and NS397 to alleviate both hyperalgesia and edema (Ndengele et al., 2008). The difference in the efficacy and potency of these gel formulations in these nociceptive models could be due to participation of PGs generated by both COX isoforms. Although COX-2 expression increased after carrageenan injection, PGs generated by both COX isoforms participate equally in the mediation of edema and hyperalgesia (Dirig et al., 1998; Svensson and Yaksh, 2002). This agrees with previous reports in which nonselective COX, selective COX-1, and COX-2 inhibitors showed antihyperalgesic effects with variable potency (Dirig et al., 1998; Yaksh et al., 2001; Padi et al., 2004; Satyanarayana et al., 2004).

Furthermore, 2-arachodonyl glycerol (AG) is oxygenated in vivo by COX-2 producing PGE2-G, which induced mechanical allodynia and thermal hyperalgesia, thus it plays a role in pain and immunomodulation (Hu et al., 2008). The data clearly demonstrates that COX-2 could act as an enzymatic switch by converting 2-AG from an antinociceptive mediator to a pro-nociceptive prostanoid. Moreover, evidence exists that central and peripheral COX-2 are equally involved in mechanical hyperalgesia, while central COX-2 is predominantly involved in thermal hyperalgesia (Okumura et al., 2008). It is clear from previous studies that COX-2 is mainly involved in the maintenance of inflammation and related hyperalgesia following carrageenan injection and pharmacological modulation by COX-2 inhibition could alleviate such conditions better than a selective COX-1 or non-selective COX-1/2 inhibitor. Thus, the results demonstrated that NIZER gel had marked and comparatively similar anti-inflammatory but better antihyperalgesic activity than VOVERAN emulgel.

Hydrogels are three dimensional hydrophilic polymer networks capable of swelling in water or biological fluids, and retaining a large amount of fluids in the swollen state (Brown et al., 2006; Satish et al., 2006; Klouda and Mikos, 2008). In the clinical assessment of a topical gel, the gelling agents and permeation enhancers used may significantly affect drug release and skin permeation, thereby altering the biological activity (Satish et al., 2006; Huang et al., 2007). Drug release from different carbopol, poloxamer, hydroxyl propyl methyl cellulose and chitosan based formulations have been investigated and such formulation had shown superior drug release and pharmacodynamic efficacy (Nanjawade et al., 2007; Klouda and Mikos, 2008). The poor bioavailability of various conventional solutions has also been overcome by the use of in-situ gel forming systems that are instilled or applied as drops at infected or inflamed sites and then undergo a sol-gel transition (Nanjawade et al., 2007; Omidian et al., 2007). Therefore, from the pharmaceutical point of view, the role of excipients in drug delivery in altering the pharmacodynamic profile of drugs can not be discarded.

In summary, the present study has shown that topically applied NIZER gel is more effective at relieving inflammation and associated inflammatory pain. Overall, the pharmacological effects of VOVERAN emulgel or NIZER gel in animal model of inflammation were broadly similar, although NIZER gel was markedly more efficacious in alleviating hyperalgesia. The emerging role of COX-2 as an inducible enzyme in various diseases and the present results further support the potential of topical use of selective COX-2 inhibitors with a better safety profile in the management of pain and inflammatory disorders.

CORRESPONDENCE TO
Dr. Satyanarayana S.V. Padi Assistant Professor, Department of Pharmacology, I.S.F. College of Pharmacy, Ghal Kalan, Moga, Punjab 142001, India. Telefax: +91-1636-236564 e-mail: ssvpadi@rediffmail.com

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r-2. Brown MB, Martin GP, Jones SA, Akomeah F.


Author Information

Satyanarayana S.V. Padi, Ph.D.
I.S.F. College of Pharmacy

Minakshi Gupta, M.Pharm
I.S.F. College of Pharmacy

Jatinder K. Kehal, M.Pharm
I.S.F. College of Pharmacy

Ashish Aggarwal, B. Pharm
I.S.F. College of Pharmacy

Neeraj Kumar, B. Pharm
I.S.F. College of Pharmacy

Rakesh K. Marwaha, M.Pharm
I.S.F. College of Pharmacy