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Enhanced anti-inflammatory effects of celecoxib from a transdermally applied nanoemulsion

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The aim of the present study was to evaluate the enhanced anti-inflammatory effects of celecoxib (CXB) from a transdermally applied nanoemulsion. The anti-inflammatory effects of an optimized nanoemulsion formulation were compared with those of conventional CXB gel and nanoemulsion gel on carrageenan-induced paw edema in rats. These tests were compared using the Dunnett test of one-way analysis of variance (ANOVA). The % inhibition value after 24 h application was significant for optimized formulation C2 (85.4%) compared with CXB gel and nanoemulsion gel ($p < 0.05$). These results suggest that nanoemulsions can be successfully used to enhance the anti-inflammatory effects of CXB.

1. Introduction

Celecoxib (CXB), a selective cyclo-oxygenase-2 (COX-2) inhibitor has been recommended for oral use for the treatment of arthritis and osteoarthritis. Long term oral administration of CXB causes serious gastrointestinal side effects (Shakeel et al. 2008a). Recent long term studies in patients with rheumatoid arthritis, osteoarthritis and acute pain have shown that instead of great benefits of oral CXB in treating such diseases, gastric irritation and ulceration have not yet been completely eliminated (Stichtenoth and Frolich 2003). The transdermal route eliminates these side effects and maintains a therapeutic concentration for a longer period of time (Shakeel et al. 2007, 2008b, c). Therefore the aim of the present study was to evaluate the anti-inflammatory effects of CXB from a transdermally applied nanoemulsion in order to eliminate its GI (gastrointestinal) side effects. Nanoemulsions are thermodynamically stable transparent isotropic dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size 10–100 nm (Shafiq et al. 2007a, b, c; Ahmed et al. 2008). The thermodynamic stability of nanoemulsions offers advantages over unstable dispersions like emulsions and suspensions (Shakeel et al. 2008d). Nanoemulsions are known to increase the skin permeation and therapeutic efficacy of many drugs (Baboota et al. 2007a, b; Shakeel et al. 2008c, 2009). This article evaluates the anti-inflammatory effects of CXB as a transdermally applied nanoemulsion and compares its therapeutic effects with conventional CXB gel and nanoemulsion gel. The nanoemulsion and nanoemulsion gel were prepared using nonirritant, pharmaceutically acceptable ingredients without using additional permeation enhancers, as components of the nanoemulsions themselves act as permeation enhancers.

2. Investigations, results and discussion

The anti-inflammatory effects of CXB in an optimized nanoemulsion formulation (C2) were compared with those of nanoemulsion gel (NGC2) and conventional CXB gel (CXB gel) formulations. The percent inhibition value after 24 h application was found to be highest for C2, i.e. 85.4% as compared to 43.7% for CXB gel. The difference compared with CXB gel was highly significant at the 1% level ($p < 0.01$). The percent inhibition value for formulation NGC2 was found to be 67.8% (Fig.). The difference was significant at the 5% level ($p < 0.05$) when comparing formulation C2 with formulation NGC2 (nanoemulsion gel). CXB in nanoemulsion and nanoemulsion gel

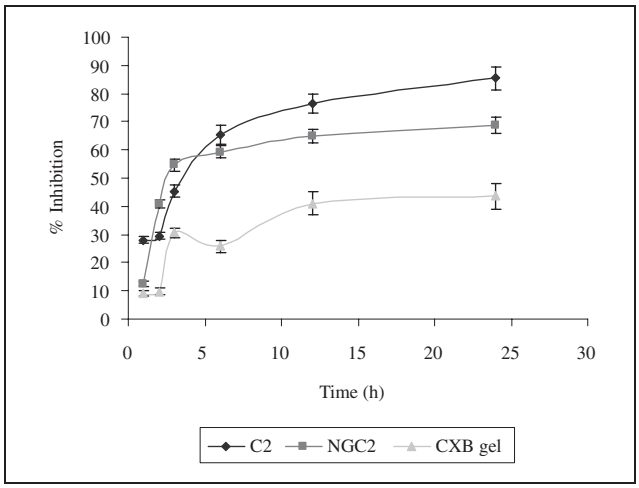

Fig.: Anti-inflammatory effects of nanoemulsion (C2), nanoemulsion gel (NGC2) and conventional CXB gel

formulations exhibited both local and systemic actions. An increase in the systemic anti-inflammatory effects of CXB leads to complete inhibition of the inflammation process. The local gradient coupled with the increase in the amount of CXB absorbed over 24 h caused almost complete inhibition of inflammation by the nanoemulsion formulation.

The enhanced anti-inflammatory effects of nanoemulsion formulation C2 could be due to the enhanced permeation of CXB through the skin (Shakeel et al. 2009).

3. Experimental

3.1. Materials

CXB was kindly donated by Ranbaxy Research Laboratories (India). Propylene glycol mono caprylic ester (Sefsol 218) and diethylene glycol monoethyl ether (Transcutol-P) were kindly donated by Nikko Chemicals (Japan) and Gattefossé (France) respectively. Glycerol triacetate (Triacetin) was purchased from E-Merck, India. Polyoxy-35-castor oil (Cremophor-EL) was purchased from Sigma Aldrich, USA. All other chemicals used in the study were of analytical reagent (AR) grade.

3.2. Preparation of nanoemulsion and nanoemulsion gel

Various nanoemulsions were prepared by the aqueous phase titration method. The optimized nanoemulsion (C2) was prepared by dissolving 2% w/w of CXB in 15% w/w of a combination of Sefsol-218 and Triacetin (1 : 1). Then 35% w/w of a mixture of Cremophor-EL and Transcutol-P was added slowly in the oil phase. Then a sufficient quantity of distilled water was added to bring the final preparation to 100% w/w (Shakeel et al. 2009).

The nanoemulsion gel was prepared by dispersing 1% w/w of Carbopol-940 in a sufficient quantity of distilled water. This dispersion was kept in the dark for 24 h for complete swelling of the polymer. 2% w/w of CXB was dissolved in 15% w/w of a mixture of Sefsol-218 and Triacetin (1 : 1). The CXB solution was added slowly to the polymer dispersion. 0.5% w/w of triethanolamine (TEA) was added to this mixture to neutralize Carbopol-940. Then 35% w/w of a mixture of Cremophor-EL and Transcutol-P (1 : 1) was added slowly. Then the remaining quantity of distilled water was added to bring the final preparation to 100% w/w (Shakeel et al. 2008a). The compositions of the nanoemulsion and nanoemulsion gel are given in the Table.

3.3. Preparation of conventional CXB gel

The conventional CXB gel was prepared by dispersing 1 g of the Carbopol-940 in a sufficient quantity of distilled water (Baboota et al. 2006). After complete dispersion, the Carbopol-940 solution was kept in the dark for 24 h for complete swelling of the polymer. Then 2 g of CXB was dissolved in 10 g of polyethylene glycol-400 (PEG-400). This drug solution was added slowly to the aqueous dispersion of carbopol-940. Then 10 g of isopropyl alcohol (IPA), 10 g of propylene glycol (PG) and 0.5 g of triethanolamine (TEA) were added to achieve homogeneous dispersion of the gel. Then the remaining quantity of distilled water was added to make 100% of gel (Table).

Table: Compositions of nanoemulsion (C2), nanoemulsion gel (NGC2) and conventional CXB gel (CXB gel)

Ingredients	C2	NGC2	CXB gel
CXB (% w/w)	2.0	2.0	2.0
Carbopol-940 (% w/w)	–	1.0	1.0
Sefsol 218 (% w/w)	7.5	7.5	–
Triacetin (% w/w)	7.5	7.5	–
Cremophor-EL (% w/w)	17.5	17.5	–
Transcutol-P (% w/w)	17.5	17.5	–
IPA (% w/w)	–	–	10
PEG-400 (% w/w)	–	–	10
PG (% w/w)	–	–	10
TEA (% w/w)	–	0.5	0.5
Distilled water (q.s.)	100.0	100.0	100.0

CXB = Celecoxib, IPA = Isopropyl alcohol, PEG-400 = Polyethylene glycol-400, PG = Propylene glycol, TEA = Triethanolamine, q.s. = Quantity sufficient

3.4. In vivo anti-inflammatory studies

Approval to carry out *in vivo* studies was obtained from the Institutional Animal Ethics Committee, Jamia Hamdard, New Delhi, India and their guidelines were followed for the studies. The anti-inflammatory effects of the optimized formulations were evaluated by the carrageenan-induced hind paw edema method using a method developed in Wistar rats (Winter et al. 1965). Young male Wistar rats, weighing 180–220 g were randomly divided into 4 groups each containing 6 rats: control, nanoemulsion (C2), nanoemulsion gel (NGC2) and conventional CXB gel. The animals were kept under standard laboratory conditions, at a temperature of $25 \pm 1^{\circ}C$ and relative humidity of $55 \pm 5\%$. The animals were housed in polypropylene cages, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. Dose for the rats was calculated on the basis of the weight of the rats according to the surface area ratio (Shakeel et al. 2008a). The abdominal region of the rats was shaved 12 h before starting the experiments except in the control group. Nanoemulsion, nanoemulsion gel and CXB gel formulations were applied to the shaved abdominal region of all animals (except the control group) half an hour before subplantar injection of carrageenan in the right paw. Paw edema was induced by injecting 0.1 ml of a 1% w/v homogeneous suspension of carrageenan in distilled water. The volume of the paw was measured at 1, 2, 3, 6, 12 and 24 h after injection using a digital plethysmometer (Ugo Basile, Italy).

The amount of paw swelling was determined at each interval and expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group using the equation:

$$\% \text{ Inhibition} = \frac{\% \text{ Edema (control)} - \% \text{ Edema (formulation)}}{\% \text{ Edema (control)}} \times 100$$

Results of anti-inflammatory effects were compared using Dunnett's test of one-way analysis of variance (ANOVA).

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