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Pharmacodynamic evaluation of proniosomal transdermal therapeutic gel containing celecoxib

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ABSTRACT: A low dose proniosomal gel containing celecoxib was developed for the treatment of osteoarthritis. All the prepared formulations were subjected to physicochemical evaluations and anti-inflammatory studies. The entrapment was $> 90\%$. The vesicle shape was determined with the help of transmission electron microscopy. The vesicle size, size-distribution, and polydispersity studies were performed using photon correlation spectroscopy. Anti-inflammatory studies were performed using the rat hind-paw oedema induced by carrageenan (1% w/v). The selected proniosomal gel (N1LE3) produced 100% inhibition of paw oedema in rats up to 8 h after carrageenan injection. It produced 95% and 92% inhibition after 12 h and 24 h, respectively. These results indicate that proniosomes are a promising carrier for the transdermal delivery of celecoxib. Thus celecoxib can be formulated into a low dose proniosomal gel that can save the recipient from the adverse effects of large doses.

KEYWORDS: proniosomes, NSAID, osteoarthritis, TDDS

INTRODUCTION

Niosomes are nonionic surfactant vesicles having potential applications in the delivery of hydrophobic as well as hydrophilic drugs. In contrast to conventional dosage forms, the drug release rate and/or the affinity for the target site can be adjusted by modifying the composition or surface of these carriers. Niosomes can also act as drug reservoirs. Proniosomes can be converted into niosomes by agitation in water for a short time. It presents a useful vesicle-delivery concept with potential to deliver drugs via the transdermal route[1]. This would be possible if proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions[2].

The stratum corneum, the outermost layer of the skin, constitutes a large diffusion barrier inhibiting the permeation of bioactive materials. Vesicular systems are considered very promising in overcoming this permeation barrier by acting as permeation enhancers or even as vehicles for bioactive materials[3]. They can be used as vehicles for controlled percutaneous drug delivery[4].

Osteoarthritis is the most common of the rheumatic diseases and is the principal source of pain and disability in the elderly. The prevalence of this disease increases with age. Non-steroidal anti-inflammatory drugs (NSAIDs) are the drugs of choice for the management of a variety of acute and chronic inflammation and chronic degenerative orthopathies[5]. The major drawback to anti-inflammatory drug use is the preponderance of gastrointestinal (GI) side effects encountered when administered orally. Side effects are generally recognized to arise as the drug interferes with the biosynthesis of prostaglandins and other arachidonic acid metabolites in the gastric mucosa. The most common GI adverse effects include GI perforations, ulcerations, and bleeding, all of which may require hospitalization[6]. The NSAID mediated toxicity is often dose related[7]. Thus reduction in serum concentration should also lessen the risk of potentially serious systemic adverse effects secondary to NSAID induced prostaglandin inhibition such as acute renal insufficiency, nephritic syndrome, NSAID gastropathy, prolonged bleeding time, and fluid retention. This creates a need for an alternative route of administration that bypasses the gastro-hepatic metabolism of the drug. The transdermal route is such an alternative.

Celecoxib (CXB) is a nonsteroidal anti-inflammatory agent that exhibits anti-inflammatory, analgesic, and antipyretic activities with selective cyclooxygenase-2 inhibitory activity and is used in the treatment of osteoarthritis. It inhibits the cyclooxygenase-2 isoform, decreasing the production of prostaglandin E2 at inflammation sites. Selective cyclooxygenase-2 (COX-2) inhibitors are known to inhibit the production of vascular prostacyclin (PGI2),

Table 1 Composition and appearance of different proniosomal formulations (drug = 100 mg).

Formulation code	Ingredients (mg)		
	Span	Cholesterol	Soya Lecithin
N1LE2	1800	200	1800
N1LE3	1800	200	900
N1LE4	1800	400	1800
N2LE2	1800	200	1800
N2LE3	1800	200	900
N2LE4	1800	400	1800

an inhibitor of platelet aggregation and a vasodilator.

The aim of the present study was to develop a low dose transdermal drug delivery system (proniosomal gel) for CXB which undergoes hepatic first-pass metabolism and shows low bioavailability. CXB possesses all the characteristics that make it an ideal candidate for developing into transdermal system, namely, high partition coefficient ($\log P = 3.683$)[8], low molecular weight (MW = 381.38), and low bioavailability (22–40%)[9].

MATERIALS AND METHODS

The materials used include celecoxib (Ranbaxy Pharmaceuticals, India), span 40 & 60 (S.D. Fine Chem, Mumbai), cholesterol (G.S. Chemical Testing Lab. & Allied Industries, New Delhi), methanol (S.D. Fine Chem), ethanol (95%), isopropanol (Merck, Mumbai), polyethylene glycol-400 (Central Drug House), ammonia solution (Thomas Baker (Chemicals), Mumbai), potassium dihydrogen orthophosphate (Central Drug House), sodium hydroxide pellets (E-Merck) and other chemicals were of analytical reagent grade.

Formulation of proniosome gel

Proniosomes were prepared by mixing surfactant (Span 40 – N1 and Span 60 – N2), alcohol (ethanol or isopropyl alcohol, 2.5 ml) and CXB (100 mg) in a dry glass tube. The open end of the glass tube was covered with a lid to prevent the loss of solvent and warmed in a water bath at 60–70 °C until the surfactant dissolved completely. Isotonic phosphate buffer (IPB, pH 7.4, 1.6 ml) was then added as an aqueous phase and warmed on the water bath until a clear solution was formed which on cooling was converted into proniosome with the appearance of a translucent amber gel (Table 1). The ultimate ratio of surfactant:alcohol:aqueous phase was 5:5:4 w/w/w.

The hydroxypropyl methyl cellulose (HPMC) gel (4% w/v in ethanol) was selected as a suitable base to incorporate proniosome into a formulation. The polymeric gel of HPMC (1 g) was mixed with 1 g of optimized (based on physicochemical studies) formulation to get proniosome gel formulation for in vitro and in vivo studies.

Evaluation of proniosome formulation size and size distribution

Vesicle size and size distribution, both of which are important for the topical administration of vesicles[10], were determined by photon correlation spectroscopy (Photocor FC, USA). This technique is ideal for measuring the nanoparticle size, diffusion coefficient, and molecular weight of polymers in solutions. A diluted (10 mg of proniosomal gel in 100 ml IPB) suspensions of niosome (hydrated proniosome) that became a clear solution after sonication was used to determine the size and size distribution.

Microscopic evaluation

For microscopic evaluation, proniosome formulation was hydrated with IPB and a thin layer was spread on a slide. After placing a cover slip, it was observed under a light microscope (Motic, Japan) and photomicrographs were taken at suitable magnification.

The morphology and structure of niosomes formed after hydrating proniosome formulations were observed using a Morgagni 268D transmission electron microscope (TEM, Fei 155 Company, Netherlands) operating at 70 kV capable of point-to-point resolution. A combination of bright field imaging at high magnification and diffraction modes were used to reveal the morphology and size of niosomes. Proniosome formulations were suitably diluted with MilliQ water. One drop of diluted sample was applied on a carbon-coated grid, stained with one drop of 3% phosphotungstic acid and allowed to dry for 30 s before observation under the electron microscope.

Entrapment efficiency

To 200 mg of proniosome formulation, weighed in a plastic centrifuge tube, was added 10 ml of IPB. The aqueous suspension was sonicated in a sonicator bath (Nirmal International, Mumbai). The niosomal dispersion was centrifuged at 25 600g at 20 °C for 50 min to separate CXB-containing niosome from the untrapped drug. The precipitate consisting of the vesicular pellets was washed three times with IPB. All the supernatants were taken and diluted with methanol. The CXB concentration was analysed. The percentage of drug entrapment was calculated as $(C_\mathrm{t} - C_\mathrm{f})/C_\mathrm{t}$, where C_t is the total concentration of CXB and C_f is the concentration of free CXB. In all

cases a CXB-free proniosome formulation was used as a blank.

In vitro skin permeation study

The permeation study of CXB from proniosome gel formulations was determined by using a Keshary-Chien type diffusion cell (vertical). The capacity of the receptor compartment was 55 ml with an area of 9.73 cm^2. Albino rats were stunned and sacrificed, and skins were excised from the abdominal region and stored in a deep freezer (-20 °C) until use. On the day of the experiment, the skin was brought to room temperature and then treated with 0.32 M ammonia solution for 1 h and hair and fat were removed manually. The skin was washed with distilled water and examined for cuts or holes if any.

For stabilization, the skin was mounted between the two half-cells of the apparatus and the extra skin was cut and trimmed to prevent lateral diffusion. The stratum corneum side of the skin faced the donor compartment whereas the dermis faced the receiver compartment and the apparatus was assembled with springs. Both compartments were filled with IPB and methanol (60:40). The receiver fluid was stirred with a magnetic bead at a speed of 600 rpm and the assembled apparatus was placed in an incubator at 37 ± 0.5 °C. The receiver fluid was replaced every 15 min to stabilize the skin. It was found that the sample at 4 h and beyond showed negligible UV absorption indicating complete stabilization of the skin.

After complete stabilization the donor compartment was filled with 2 g of proniosome formulation (which is equivalent to 42–52 mg of CXB). The top of the diffusion cell was covered with paraffin paper. The receiver fluid was stirred with a magnetic bead at a speed of 600 rpm and the assembled apparatus was placed in an incubator at 37 ± 0.5 °C.

The permeation of niosome suspensions of CXB (diluted proniosome formulations) was determined by the side-by-side diffusion cell. The niosome suspension was prepared by agitating 200 mg of proniosome gel (N1LE3) in 10 ml of IPB in a glass tube. The skin was mounted between the two half-cells of the diffusion cell. After complete stabilization the sonicated suspension was used to fill the donor compartment. The receiver compartment was filled with IPB and methanol (60:40). The receiver fluid was stirred with a magnetic bead at a speed of 600 rpm and the assembled apparatus was placed in an incubator at 37 ± 0.5 °C. Samples were withdrawn after various times up to 24 h and analysed.

Stability of proniosome gel

Stability of the proniosomal formulations was determined by storing in glass Petri dish covered with aluminium foil at room temperature (30 ± 2 °C) and in a refrigerator (4 ± 2 °C).

After 5, 15, and 30 days they were observed visually and under optical microscope for changes in consistency and appearance of drug crystals.

Pharmacodynamic studies (anti-inflammatory studies)

Approval to carry out studies in vivo was obtained from the institutional animal ethics committee, Jamia Hamdard, New Delhi and their guidelines were followed throughout the studies. The anti-inflammatory activities of proniosomal gel formulation were evaluated using the carrageenan-induced hind-paw oedema method[11]. The oedema of the paw was measured with the help of a digital plethysmometer (7140, UGO Basile, Italy). Young Wistar rats weighing 150–250 g were randomly divided into three groups (test, standard, and control) each containing 6 rats. Marketed preparations of CXB to be used transdermally were not commercially available. Instead, a gel of carbopol-934 (1% w/v) was prepared, in which the drug was dissolved in polyethylene glycol-400 (PEG-400, 0.1% w/v).

The rats were given free access to water and food and kept under observation for 24 h. The backsides of the rats were shaved 12 h before starting the experiment. The proniosomal gel and carbopol gel were applied on the shaved abdomen (diameter 3.5 cm) of all animals in the test and standard groups, respectively. Right hind paw oedema was induced in all three groups of animals by subplantar injection of 0.1 ml of a 1% w/v homogeneous suspension of carrageenan in distilled water. In test and standard group animals, carrageenan was injected half an hour after the application of the formulations. The control group remained untreated (no formulation was applied before/after carrageenan injection). The swelling of the injected paw was measured immediately (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h after injection using a digital plethysmometer. The amount of paw swelling was determined from time to time and expressed as the ratio of oedema to initial hind paw volume. The mean values of the ratios were determined at each time interval. Percentage inhibition of oedema produced in each group was calculated against the respective control group as %Inhibition = [%Oedema (Control) − %Oedema (Formulation)]/[%Oedema (Control)],

where (Mean oedema) = (Mean of final paw volume) − (Mean of initial paw volume), and %(Paw oedema) = [(Final paw volume) − (Initial paw volume)]/[(Initial paw volume)].

HPLC analytical method

CXB was analysed by high performance liquid chromatography (HPLC, Shimadzu, Japan). HPLC was equipped with quaternary LC-10A VP pumps, variable wavelength programmable UV-Vis detector SPD-10AVP, column oven, SCL 10AVP system controller, A rheodyne injector fitted with a 20-ml loop and Class-VP 5.032 software was used. The HPLC column used was a reverse phase 25 cm×0.46 cm ID SUPELCO 516 C18 DB, 5 µm. The whole system was kept at ambient conditions. The mobile phase was methanol/water (75:25) with a flow rate of 1.25 ml/min. The injection volume was 20 µl and the elute was analysed at 250 nm.

Statistical analysis

All drug release and skin permeation experiments were repeated three times and data were expressed as the mean ± SD. Statistical data were analysed by one-way ANOVA. A Dunette multiple comparison test and paired t-test were used to compare different formulations and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Based on the entrapment of the drug, Span 40 and Span 60 were selected as non-ionic surfactants. They also give the least leaky niosomes as these Span surfactants have the highest phase transition temperature[12]. Soya lecithin was selected over egg lecithin because the former gives vesicles of larger size, possibly due to differences in the intrinsic composition of soya and egg derived lecithin[13]. Preparations with a white semi-solid appearance were obtained with span and cholesterol. Incorporation of lecithin results in a gel-like appearance. The proniosomal formulations containing soya lecithin were arrested at the liquid crystalline compact niosomal transition point. It was not practical to arrest the liquid crystalline proniosomal stage, as has been reported for pure egg lecithin[14]. The types of alcohol affect the size of niosomal vesicles as well; ethanol gave the largest and isopropanol gave the smallest. The larger size with ethanol is due to the slower phase separation because of its greater solubility in water. The smaller size with isopropanol may be due to its branched chain[13,15].

The transdermal dose of CXB is lower than the oral dose since Transdermal dose = Oral dose (mg/day) × Bioavailability (%)[16]. The total daily maximum recommended dose of CXB for the relief of signs and symptoms of osteoarthritis is 200 mg administered as a single dose through the oral route. Taking the mean oral bioavailability as 40%[9], the calculated daily transdermal dose is 80 mg, which is much lower than the oral dose. Thus it would definitely reduce the dose related toxicity of CXB.

Table 2 Entrapment efficiency and spectroscopic characterization of different proniosomal formulations.

Code	%Entrapment*	Size (nm) (Mean ± SD)	PI $(SD/Mean)^2$
N1LE2	93.8 ± 2.1	-	-
N1LE3	93.8 ± 2.0	449.4 ± 2.7	3.7×10^{-5}
N1LE4	93.7 ± 3.5	-	-
N2LE2	92.5 ± 2.7	-	-
N2LE3	93.8 ± 1.6	317 ± 287	0.823
N2LE4	93.1 ± 2.9	-	-

* Values represent mean ± SD ($n = 3$).

The mean size, standard deviation, and polydispersity index (PI) were determined (Table 2). Vesicles of niosomes formed from N1LE3 were observed to be moderately larger than those formed from N2LE3. The niosome vesicles formed from N1LE3 had a mean size of 449.4 nm. An admirable uniformity in particle size distribution was obtained as indicated by a low polydispersity index (PI < 1), indicating the dispersions were rather polydisperse and the method of niosome formation made the vesicles fairly uniform in size[13].

Microscopic evaluation of formulation N1LE3 with the light microscope image showed niosomes and separated niosomes from proniosome gel when hydrated with IPB. The TEM images of the niosomes obtained from proniosomal formulation show that the vesicles were spherical and discrete with sharp boundaries (Fig. 1).

All the formulations showed a high value of %Entrapment ($> 90\%$, Table 2). Niosomes formed from Span 40 and Span 60 exhibited very high entrapment ($p < 0.05$). The high entrapment efficiency may be attributed to the high lipophilic nature of the drug ($\log P = 3.683$) that is expected to be partitioned almost completely within the lipid bilayers of niosomes. Hence in proniosomal formulation, the entire drug will be intercalated within the bilayers as opposed to the aqueous spaces within the gel. This result was consistent with the entrapment efficiency of levonorgestrel[13], ketorolac[17], and oestradiol[2] in proniosomes incorporated with Span 40 and 60. Increas-

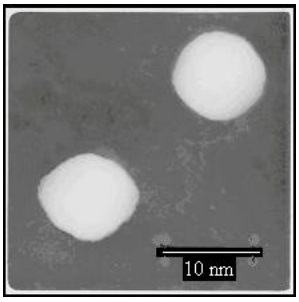

Fig. 1 TEM images of niosomes formed after hydration of proniosome gel (N1LE3) at a magnification of 44 000× showing spherical shape.

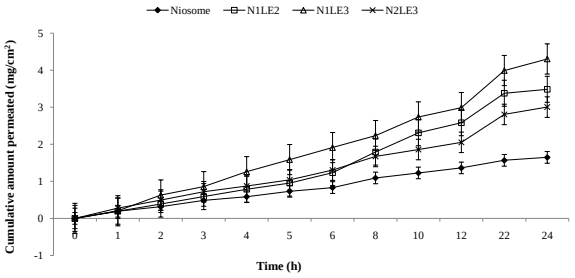

Fig. 2 Drug permeation profile of proniosomal formulations (N1LE2, N1LE3, N2LE3) and niosome suspension.

Table 3 Cumulative percentage permeated and flux of CXB.

Formulation	Cumulative % % permeated	Flux (mg/cm^2/h)
N1LE2	79.16 ± 0.14	0.15 ± 0.01
N1LE3	86.26 ± 0.42	0.17 ± 0.14
N2LE3	60.61 ± 0.32	0.12 ± 0.03
Niosome suspension	80.30 ± 0.19	0.06 ± 0.01

Values represent mean $\pm$ SD ($n = 3$).

ing the cholesterol content contributed an increase in the hydrophobicity, with subsequent reduction of vesicle size[17] thereby also reducing the entrapment efficiency. Based on the entrapment efficiency the formulations N1LE2 (93.8%), N1LE3 (93.8%), and N2LE3 (93.8%) were selected for in vitro permeation studies.

The permeation studies provide valuable information of the product behaviour in vivo since they indicate the amount of drug available for absorption. The high cumulative percentage of drug permeated (Fig. 2) may be attributed to the fact that Span 40 gives the vesicle of a larger size with a higher entrapment of drug[12]. Also, the drug leaking from the vesicles composed of Span 40 is low due to its high phase transition temperature and low permeability. No lag phase could be detected because of the low sampling time of 1 h and the permeation of CXB through rat skin was found to nearly linear indicating a constant slow release.

The N1LE2 and N1LE3 formulations showed a higher permeation (Table 3). Increasing the lecithin content in the vesicles resulted in a higher flux ($p < 0.05$). This could be explained by the minor disruption of vesicles due to the reduction in the lecithin content which leads to the leakage of free drug before fusion of the vesicles with the skin. The reverse is true upon increasing the lecithin content. Alsarra et al[17] investigated the lowering of flux of ketorolac upon decreasing the lecithin content. These results imply that inclusion of lecithin at a definite level may play a significant role in drug permeation. In addition, lecithin is reported to have a weak permeation ability[2]. No effect was observed on the transdermal delivery of CXB upon increasing the cholesterol content, in agreement with previous findings[17,18]. The permeation of oestradiol, however, increases on reducing the cholesterol content[19], indicating a different mechanism of drug transport across the skin depending on the composition of the niosomal formulation and the drug used.

The in vitro skin permeation study of niosome suspension was carried out in a side-by-side diffusion cell. This was done in order to compare the efficiencies of transdermal drug delivery through niosome suspension and proniosomal gel. The trend of amount of drug permeated from the different formulations (N1LE3 > Niosome suspension > N1LE2 > N1LE4 > N2LE3) differed from that of flux (N1LE3 > N1LE4 > N1LE2 > N2LE3 > Niosome suspension). This indicates that niosomes and proniosomes exert a distinct influence on the CXB permeation across skin. Free CXB passed through the skin as easily as CXB in niosomes, indicating that the niosome lipid bilayers are rate limiting membrane barriers for CXB. The lower flux of CXB from niosomes may be due to the considerable dilution of proniosome formulations. Another explanation is that there may be an increased leakage of free drug due to decreased stability of niosome suspension which can improve transdermal delivery of lipophilic molecules.

Stability studies were performed for the N1LE3 and N2LE3 formulations. No drug crystals were observed after one-month storage at 30 ± 2 °C or at 4 ± 2 °C. The consistency of the gel increased because of the molecular interaction of polar head groups of surfactants with the solvent and permeation of solvent into the bilayers. The solvent diffusion into the

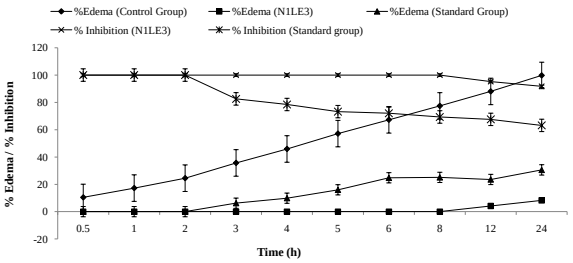

Fig. 3 Pharmacodynamic studies of proniosomal gel (N1LE3).

bilayers did not disturb the liquid crystalline structure. It instead resulted in complete bilayer formation due to the saturation of the lipid polar heads. This might have led to an increase in bilayers distance resulting in an overall increase in consistency. Loss of alcohol upon storage may have been another reason of the increase in consistency[2].

The proniosomal gel (N1LE3) completely inhibited the oedema for up to 8 h (i.e., there was no oedema in rats of test group after 8 h of carrageenan challenge), and the inhibition was 95% and 92% after 12 h and 24 h, respectively. The mean percentage oedema in the control group of Wistar rats was found to be $10 \pm 3\%$ and $100 \pm 41\%$ after 0.5 h and 24 h of carrageenan injection, respectively. There was 100% inhibition of oedema in the control group after 2 h of carrageenan injection (Fig. 3). There was only 63% of inhibition of oedema in the standard group after 24 h of carrageenan injection.

CONCLUSIONS

Permeation of CXB from proniosome gel was observed to be better than that of niosome suspension. This indicates that only proniosome gel appears to proficiently deliver CXB by the transdermal route. A significant amount, for the preparation of proniosomal gel, of Span 40 and lecithin in ethanol was needed to enhance CXB permeation across skin. The literature suggests that the direct transfer of the drug from the vesicles to the skin and/or the permeation enhancement effect of the nonionic surfactants may contribute to the mechanism of drug permeation enhancement by proniosomal formulations. Proniosomes may be a suitable carrier for CXB or other drugs due to their ease of production and the fact that they do not require the use of pharmaceutically unacceptable additives. It was found that N1LE3 formulation has the best effective combination of surfactant, alcohol, and aqueous phase among the formulations studied for further development of proniosomal gel into a transdermal delivery of CXB for the treatment of osteoarthritis. CXB can be formulated into low dose proniosomal gel for transdermal delivery that can save the recipient from the harm of large doses with improved bioavailability (by by-passing the hepatic first metabolism) and can be recommended for further pharmacokinetic and pharmacodynamic studies in suitable animal models.

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