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Development and characterization of a novel controlled release drug delivery system based on nanostructured lipid carriers gel for meloxicam

S. Khurana [a], N.K. Jain [b], P.M.S. Bedi [a,*]

[a] Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143104, India
[b] Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar 470003, India

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ABSTRACT

Aim: The main objective of the current investigation was to develop nanostructured lipid carriers (NLC) based gel for the enhancement of transdermal absorption of meloxicam (MLX) to achieve local as well as systemic drug action without concurrent gastrointestinal toxicity.
Main methods: NLC gel containing MLX was prepared and characterized for particle size, polydispersity, zeta potential, pH, rheology, entrapment efficiency, occlusion factor, and thermal behavior. In vitro drug release, in vitro skin permeation and deposition studies were carried out using Franz diffusion cells. Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) of MLX-NLC gel treated stratum corneum (SC) were undertaken to get an insight into the skin permeation enhancement mechanism of MLX-NLC gel. Toxicity potential of the developed gel formulation was assessed by in vitro hemolysis and histopathological examinations. The rat paw edema test was performed to evaluate the anti-inflammatory activity of MLX-NLC gel.
Key findings: MLX-NLC gel demonstrated sustained release and enhanced the skin permeation and deposition of meloxicam especially into the dermis in comparison to meloxicam gel (control). MLX-NLC had an impact on the barrier properties of the skin and acted via protein and lipid modifications in the stratum corneum. MLX-NLC gel turned out to be hemocompatible, non-irritant, and non toxic with significant anti-inflammatory activity.
Significance: The results suggest that NLC gel could be a promising carrier for the transdermal delivery of meloxicam.

Introduction

The development of transdermal drug delivery system continues to be challenging due to the inherent barrier attributes of the stratum corneum (SC), the outermost layer of the skin that prevents percutaneous absorption of drugs. In recent years, greater attention has been centered on the use of nano to submicron-sized lipid based carrier systems. Solid lipid nanoparticles (SLN) composed of physiological and biocompatible solid lipid core have gained a lot of attention as drug carriers for topical/transdermal use. They have demonstrated several advantages over polymeric nanoparticles, microemulsion, and liposomes such as good local tolerability, efficient incorporation of lipophilic drugs, more flexibility in modulating the release of the drug and improved skin targeting potential (Muller et al., 2000; Mehnert and Mader, 2001; Utreja and Jain, 2001). Nevertheless, there are two major potential issues, such as drug expulsion during storage and limited drug payload that are linked to the limited use of SLN. Alternatively, nanostructured lipid carriers (NLC) have been introduced as the second generation of lipid nanoparticles to overcome the major limitations of SLN. They are produced by replacing a part of the solid lipid of the SLN with a spatially different liquid lipid. Compared to SLN, NLC have an imperfect lipid matrix that offers enough space to accommodate the drug molecules and prevent/minimize drug expulsion during storage (Khurana et al., 2009).

Transdermal application of NLC is regarded as an attractive strategy since the adhesion of nanosized NLC to the skin surface provide an occlusive effect to the skin. The occlusive effect can eventually lead to an increase in skin hydration and can promote the deposition of drugs into the viable skin by reducing corneocytes packing and widening inter-corneocytes gaps (Muller et al., 2002; Schäfer-Korting et al., 2007). Additionally, the components of NLC such as lipid and surfactants can also function as permeation enhancers by reducing the barrier properties of SC and consequently increasing the permeation of drug through the skin (Guo et al., 2012). Since NLC dispersion possesses low viscosity and is consequently inconvenient to use on the skin, they must be converted into gel to ease the application and to prolong residence time on the skin.

Meloxicam, a non-steroidal anti-inflammatory drug (NSAID), is widely used in the symptomatic treatment of joint disorders (osteoarthritis and rheumatoid arthritis). Its use is associated with various

* Corresponding author at: Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar 143104, Pb., India. Tel./fax: +91 183 2258819.
E-mail address: bedi_preet@yahoo.com (P.M.S. Bedi).

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gastrointestinal side effects similar to other NSAIDs (Distel et al., 1996; Lanes et al., 2000). Transdermal administration of meloxicam can be specifically advantageous for the management of arthritic conditions as it would bypass the gastrointestinal tract and would allow an increased level of drug locally. Contemplating, all these points, we aimed to develop NLC-based gel for the transdermal delivery of meloxicam. NLC gel was suitably characterized for particle size, zeta potential, pH, entrapment efficiency, rheology, drug release properties, in vitro skin permeation and deposition, in vivo toxicity, and in vivo pharmacodynamic activity. Furthermore, the effect of MLX-NLC gel on the biophysical status of skin was also investigated to get an insight into its deposition enhancement mechanism.

Materials and methods

Materials

Meloxicam was received as a gift sample from M/s Lupin Pharmaceuticals Ltd., Goa, India. Cetyl palmitate, Caprylic acid, propylene glycol (PG), Tween 80, polyethylene glycol 400 (PEG 400), and triethanolamine were purchased from S. D. Fine Chemicals, Mumbai, India. Carrageenan, Carbopol 940, Trypsin (Type III bovine), HPLC grade methanol, water and phosphoric acid were purchased from Sigma-Aldrich, Mumbai, India. Dialysis membrane (MWCO 12-14,000), Triton X-100 was purchased from Hi-Media, Mumbai, India. All other chemicals were of the analytical grade and used as received.

Methods

Preparation of nanostructured lipid carriers gel (NLC gel)

The optimized plain-NLC dispersion was prepared using cetyl palmitate and caprylic acid as the lipid phase (matrix material) by microemulsion template strategy following the procedure previously described by our group (Khurana et al., 2010). A calculated volume of filtered water followed by Tween 80 and propylene glycol was added to the melted lipid phase at 65 °C under magnetic stirring to form transparent microemulsion (Table 1). Finally, NLC were produced by simply cooling the formed warm microemulsion precursor to room temperature in the same container. For the preparation of nanostructured lipid carrier gel (plain-NLC gel), Carbopol 940 was completely dispersed in the NLC dispersion with constant stirring. Thereafter, triethanolamine was added drop wise to adjust the pH (6.0) and to promote gelation. NLC gel formed was allowed to stand overnight to remove the entrapped air. Following the same method, meloxicam loaded nanostructured lipid carrier gel (MLX-NLC gel) was prepared after replacing a definite amount of the lipid phase by MLX (Table 1). As a control, meloxicam gel (MLX-gel) was prepared by dispersing Carbopol 940 to the solution of meloxicam in water containing Tween 80 and PG under stirring followed by neutralization with triethanolamine (Table 1).

Physico-chemical characterization

The particle size analysis and polydispersity index (PI) determination were carried out by photon correlation spectroscopy (PCS) with a Malvern Nanosizer ZS (Malvern Instruments, UK). NLC gel was diluted with distilled water prior to measurements. Zeta potential (ZP) was measured in a Malvern Nanosizer ZS (Malvern Instruments, UK). The morphology of NLC gel was examined by Transmission Electron Microscopy (TEM) (Morgagni 268D, FEI, Holland). The pH measurements were carried out using a calibrated digital pH meter (Hanna instruments HI 9321, Michigan, USA).

Drug entrapment efficiency (E.E)

The percentage of drug entrapped into NLC gel was determined (Utreja et al., 1999) after removing the free drug from NLC gel by ultra dialysis at 4 °C using dialysis bag (Sigma, USA; MWCO 12,000–14,000). The dialyzed formulation was then lysed using Triton-X100 (0.1% *v/v*) and subsequently analyzed for the amount of drug entrapped (A_2). The percent of drug entrapment efficiency (% E.E) was computed by using Eq. (1)

$$E.E(\%) = (A_2/A_1) \times 100 \quad (1)$$

where A_1 is the total amount of drug added in the formulation. The concentration of drug was determined by UV spectrophotometry (Hitachi U-2800 spectrophotometer, Tokyo, Japan) at 362 nm.

Thermal behavior

Differential scanning calorimetry analyses were performed using a Mettler DSC 821e differential scanning calorimeter (Mettler Toledo, Gieβen, Switzerland) to study the thermal characteristics of NLC gel. Weighed amounts of the samples (approximately 1–3 mg based on lipid content) were scanned in 40 μl aluminum pans at heating rate of 5 K/min over a temperature range of 25–85 °C. An empty standard aluminum pan was used as a reference. The DSC parameters were evaluated using STARe Software (Mettler Toledo, 821e, Switzerland). The percent of crystallinity index (CI %) was calculated according to the Eq. (2) (Washington, 1989).

$$CI(\%) = \frac{\Delta H_{sample}}{\Delta H_{bulk\ \ lipid} \times C_{lipid\ phase}} \times 100 \quad (2)$$

Where ΔH_{sample} and $\Delta H_{bulk\ lipid}$ are the measured enthalpy of bulk lipid and sample, respectively. $C_{lipid\ phase}$ is the concentration of lipid phase in the sample.

Occlusion factor

The occlusive properties of NLC gel were evaluated by in vitro occlusion test suggested by de Vringer and de Ronde (1995). Beakers (100 ml) with a diameter of 4.9 cm were filled with 50 ml of water and covered with a filter paper (Whatman number 6, cutoff size: 3 μm, USA). 250 mg of each gel formulation was applied uniformly with a spatula on the filter surface (18.8 cm^2) and the beakers were subsequently stored at 32 ± 0.5 °C (to mimic the temperature of the skin surface) for 48 h. The weight of water remaining in the beakers was weighed at 6, 24, and 48 h. The beaker covered with a filter paper was used as a control. The occlusion factor (F) was computed by the following Eq. (3)

$$F = (A - B/A) \times 100 \quad (3)$$

Table 1
Composition of the developed nanostructured lipid carrier gel (NLC-gel) and drug-gel.

Formulation	Meloxicam (mg)	Cetyl palmitate (mg)	Caprylic acid (mg)	Tween 80 (ml)	Propylene glycol (ml)	Carbopol 940 (mg)	Water (ml)
Plain-NLC gel	–	65	35	0.2	0.1	5	0.7
MLX-NLC gel	5	61.75	33.25	0.2	0.1	5	0.7
MLX- gel	5		–	0.2	0.1	5	0.7

Plain-NLC gel, nanostructured lipid carriers gel; MLX-NLC gel, nanostructured lipid carriers gel loaded with meloxicam; MLX-gel, meloxicam-carbopol gel.