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A novel nano-carrier transdermal gel against inflammation

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ABSTRACT

The objective was to develop a stable, reproducible and patient non-infringing novel transdermal drug delivery system "*nano-carrier transdermal gel*" (NCTG) in combination of partial dose replacement of diclofenac diethylamine (DDEA) by curcumin (CRM). The drug content of gel was 99.30 and 97.57% for DDEA and CRM. Plasma samples were analyzed by liquid chromatography with triple-quadrupole tandem mass spectrometer (LC–MS/MS). Data were integrated with Analyst™ and analyzed by *WinNonlin*; stability parameters were analyzed using Tukey–Kramer multiple comparison test. Its average skin irritation scored 0.49 concluded to be non-irritant, safe for human use and in vivo studies revealed significantly greater extent of absorption and highly significant inhibition (%) of carrageenan induced paw edema. The results also demonstrated that encapsulation of drugs in nano-carrier increases its biological activity due to superior skin penetration potential. Hence, a novel once day transdermal gel of nano-carrier (nano-transfersomes; deformable vesicular) is achieved, to increase systemic availability, subsequent reduction in dose and toxicity of DDEA was developed for the treatment of inflammation.

1. Introduction

In 1952, after salicylic acid, phenylbutazone first non-steroidal anti-inflammatory agent (NSAID) and a more\decade later, viable compounds (mefenamic acid, ibuprofen, and indomethacin) were introduced (Sallmann, 1976). This point was a key to begin an aimed at developing a novel anti-inflammatory drug which should be better in activity, tolerability or both (Sallmann, 1986). The concept transdermal drug delivery system (first seriously recommended by Dr. Alejandro Zaffaroni in early 1970s) is to ensure that compounds are delivered to the systemic circulation, in spite of stratum corneum at a specific rate (Ahad et al., 2009; Leonard et al., 2007). These conclusions have substantiated design the diclofenac diethylamine (DDEA) for efficient transdermal delivery in symptomatic relief (pain\inflammation\musculoskeletal disorders) and its amphiphilic nature and improved skin permeation rate is the reason for reportedly used in dermal application than other salts of diclofenac [other idyllic characteristics such as poor bioavailability (40–60%)] which mandates its transdermal drug delivery. Another, curcumin (anti-bacterial\neoplastic and anti-inflammatory) a principal component of turmeric, is a hydrophobic poly phenol and used for centuries in Asian countries as a spice and also an herbal anti-inflammatory agent (Chaudhary et al., 2013).

The specific goals of the research were to develop a novel nano-carrier transdermal gel (NCTG) containing DDEA and CRM using gelling agent (Carbopol-934P) (Chaudhary et al., 2011) without permeation enhancer and its evaluation (characterization and in vivo studies). To investigate the usefulness of DDEA on the transdermal along with CRM; resulting, a once a day NCTG (as a transdermal therapeutic system) was developed with both drugs for the local treatment of inflammation.

2. Materials and methods

Diclofenac diethylamine (DDEA; pure 100.2%, w/w) was procured from Wings Pharmaceuticals, Pvt. Ltd. (Baddi, Himachal Pradesh, India). Curcumin (CRM purity 68.57%, w/w), sodium methyl paraben and sodium propyl paraben were received as gift samples from Arbro labs (New Delhi, India). Sodium cholate and Phospholipid 90G were purchased by Thomas Baker (Chemicals) Ltd. (Mumbai, India) and Phospholipid GmbH, Nattermannallee, Cologne, (Germany) respectively. Carbopol 934P was obtained from Ranbaxy Research Lab. (Gurgaon, Haryana, India). Ethanol and propylene glycol (PG) were supplied by Changshy Yangyuan Chemical (Suzhou, Jiangsu, China) and Loba Chemie Pvt. Ltd. (Mumbai, Maharashtra, India).

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2.1. Animals

Male Wistar rats (weighing 180–300 g) approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) of Jamia Hamdard, Hamdard University (Protocol approval No. 535) and were used in the study.

2.2. Nano-carrier transdermal gel

The nano-carrier i.e. nano-transfersomes (lipid:surfactant ratio 11.90, weight of lipid and surfactant based on drug: lipid ratio 600.00 and 20 min sonication time) of DDEA and CRM were prepare by conventional sonication method (Chaudhary et al., 2013). The optimized nano-transfersomes are incorporated in the gelling base [Carbopol934P (0.84%) was used to prepared nano-carrier transdermal gel (NCTG); structure of gel may retard the leakage of the drug from the vesicles for a controlled drug release] to achieve better rheological and tissue compatibility (Chaudhary et al., 2011). The designed quantity of the gel base, sodium methyl paraben and sodium propyl paraben were added into water and kept overnight for complete humectation of the polymer chains. The required volume of optimized preparation (transdermal dose) was added to the hydrated base mixture with stirring and gelling was induced by neutralizing the dispersion solution using sodium hydroxide (pH 6.2–6.8).

2.3. Physical and pH evaluation

The prepared nano-carrier transdermal gel (NCTG) was evaluated for its cosmetic qualities and its was recorded with a glass microelectrode (Mettler instruments, Giessen, Germany) by bringing it in contact with the gel and allowing it to equilibrate for 1 min. The experiment was performed in triplicate to check for the neutralization of gel. A modified apparatus (Mutimier et al., 1965) consisting of two glass slides containing gel in between, with the lower side fixed to a wooden plate and upper one attached to a balance by a hook was used to determine spreadability, which was calculated using the following formula:$S = m \times \frac{1}{t}$where S represents the spreadability (g/s), m is the weight in pan (g), l is the fixed distance moved by the slide and t is the time.

2.4. Rheological

Gel apparent viscosity determined using a Brookfield R/S plus cone and plate Rheometer with spindle C50-1, (Brookfield Engineering Lab. Inc.). The shear rate was fixed at 01 rpm/s (50 rpm/50 s) at 25 °C after varying the rate (0.5–3.0 rpm/s) and all measurements were performed in triplicate.

2.5. Drug content and release profile

Ex vivo studies were carried out through rat skin using an automated transdermal diffusion cell sampling system (SFDC 6, LOGAN Instruments, NJ, USA) and drug content (DDEA and CRM) was determined by analyzing the concentration in gel using HPLC method (Chaudhary et al., 2012).

2.6. Release kinetics

To ascertain the release kinetics of the NCTG, the ex vivo diffusion data were applied to zero order, first order, Higuchi kinetics models and Korsmeyer–Peppas equations used to evaluate the drug release mechanism.

2.7. Skin irritation studies

It is desirable to give consideration to the skin compatibility of the drug and other ingredients of the transdermal therapeutic system (TTS). Usually, skin compatibility of TTS is determined through different tests like cytotoxicity, primary skin irritation studies and repeat-insult patch testing etc., as the formulation stays on to the skin for a duration ranging from 24 h to days. Thus it is imperative to carry out the compatibility test for skin irritancy. The irritancy of different formulations was determined in rats (180–200 g) based on the method (Draize et al., 1994). The intact and abraded skin of rats was used for assessing any kind of skin irritation arising out of the application of the transdermal preparation. The animals were divided into six groups of six animals each. The animals were housed in an air-conditioned room (20 °C) and hair on an area of 1 cm^2 of the back of all animals was trimmed short, 24 h before the beginning of the test. They were grouped equally for intact (Group I, III, V) and abraded (Group II, IV, VI) skin for each formulation respectively. For Group II, IV and VI the area was abraded by making four epidermal scratches with the help of surgical blade (two scratches were perpendicular to the other two). To the Group I and II plain drug solution was applied, Group III and IV received nano-carrier transdermal gel (NCTG) of DDEA and CRM and marketed gel (MG) of DDEA was applied to Group V and VI, respectively. Three squares were drawn on each side of the back of each animal and 0.5 ml/0.5 g of each formulation was applied on each square. The treated animals were protected by using nylon mesh, supported by the plastic squares having small pores, kept above the treated area. After exposure for 24 h, the test substance was removed and exposed skin was scored for the formation of edema (graded 0–4) and erythema graded (0–4). The areas were also examined after 72 h for any sign of erythema, escar formation and edema (final score represented an average of 24 and 72 h readings) with scale as follows: zero for no edema and erythema, one for very slight edema and erythema, two for slight edema and well defined erythema, three for moderate edema and erythema and four for severe edema and erythema (Draize et al., 1994).

2.8. In vivo studies

2.8.1. Pharmacokinetic study

The animals were kept under standardized conditions in clean cages with free access to food and water. The animals were acclimatized to laboratory conditions over the week before the experiment. The blood sampling was carried out for 48 h and blood sampling was done at the decided time periods.

2.8.1.1. Study design. Rats were divided randomly into four groups of six animals each. About 10 cm of skin was shaved on the abdominal side of rats in each group. Before application of the formulations, rats were kept under observation for 24 h for any untoward effect of shaving. They were fasted over this period and dose of the rats calculated based on the body weight of the rats was summarized in Table 1. The Group I (served as control; no treatment) was used for separation of drug free plasma and to the Group II calculated dose of nano-carrier transdermal gel (NCTG) was applied. Group III received calculated dose (as per the label claim) of marketed gel (MG) which was applied three times in a day at an equal interval of 8 h and calculated dose of plain curcumin gel (PCG) was applied to the Group IV. The rats were anesthetized using chloroform and blood samples (~0.5 ml each) were drawn through tail vein during each sampling period at the following time interval: 0, 1, 3, 4, 5, 6, 8, 12, 16, 20, 24, 36, 48 h after dose application in micro-centrifuge tubes having EDTA solution as an anticoagulant. The rats were started to feed after 6 h of dose application. The blood samples were centrifuged at 5000 rpm for 10 min and the