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Original Article

In vivo study of clobetasol propionate loaded nanoemulsion for topical application in psoriasis and atopic dermatitis

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ABSTRACT

Objectives: Optimized formulations were subjected to various *In vivo* studies like anti-inflammatory activity, Nickel induced dermatitis and irritation study. Clobetasol propionate (CP) has anti-inflammatory, immunomodulatory, and antiproliferative activity. The aim of the present work was to test the hypothesis that the addition CP in nanoemulsions would result in enhancement CP delivery and leading to better antipsoriatic activity.
Materials and methods: Nanoemulsions were prepared by aqueous phase titration method, using eucalyptus oil, Tween 20, ethanol, and distilled water as the oil phase, surfactant, co-surfactant and aqueous phase, respectively.
Results and discussion: We developed a topical O/W nanoemulsion in which drug is incorporated in disperse phase of oil and evaluated its efficacy against different types of *in vivo* studies. It was also found that the significantly increased their anti-inflammatory activity. It was reported that CP-loaded nanoemulsion significantly increased NTPDase (Nucleoside triphosphate diphosphohydrolases) activity in lymphocytes. This membrane protein is responsible for the hydrolysis of extracellular ATP (Adenosine triphosphate) which is responsible for cell proliferation, differentiation and inflammatory processes. *In vivo* irritation studies did not show any irritation in spite of having high amount of surfactant.
Conclusion: On the basis of above *in vivo* study we conclude that developed nanoemulsion is safe for human use because it has good anti-inflammatory action and did not show any irritation to the skin. All though nanoemulsion contain high amount of surfactant in comparison to cream.

1. Introduction

Topical corticosteroids are one of the oldest and most beneficial cures for dermatologic disorders. The clinical effectiveness of the tropical corticosteroids in the treatment of psoriasis and atopic dermatitis is associated with their vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative properties.[1] Despite of its clinical

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effectiveness, the application of topical glucocorticoid is restricted due to their adverse effects, such as skin atrophy, steroid acne, hypo pigmentation, allergic contact dermatitis and their poor absorption through skin.[2] The recent researches on the corticosteroids aim at developing the strategies to enhance the benefit-risk ratio of glucocorticosteroids increase their absorption and should be free from the any histopathological alteration in the structure of skin.[3] In recent years, nanotechnology have emerged as an excellent tool in the drug delivery system especially the nanoemulsions for delivering the poorly water soluble drug to the deeper layer of skin with a view to decreasing the adverse effects via decrease in dose.[4,5] Here, the particle size is reduced to its sub-micron level upto 10–200 nm,[6] to improve the absorption and therapeutic concentration of the drug in the target tissue, allowing reproducible and long-term release of the drug at the target site.[7] Clobetasol propionate (CP) is a highly lipophilic corticosteroid used for the treatment of skin disorders such as atopic dermatitis and psoriasis.[8] Therefore oil/water (O/W) nanoemulsion is prepared in which drug present in disperse phase of oil. In this system high amount of surfactant and co-surfactant is used which has irritation potential so, formulator needs to be study irritation potential before application.[9,10] The reported article of Senyigit[11] showed that the incorporation of CP into lecithin/chitosan nanoparticles induced an accumulation of CP especially in the epidermis without any significant permeation across pig ear skin. This ailment occurs because of the delayed hypersensitivity reaction, mediated by T lymphocyte to an antigen protein or a hapten linked to a protein[12] also, the extracellular ATP (Adenosine triphosphate), which is able to regulate the cell–cell interactions and play important role in the processes of cell activation, differentiation, development, proliferation, and death, as well as effector lymphocyte response.[13] NTPDase (ecto-nucleoside triphosphate diphosphohydrolase; CD39) is an integral membrane protein that metabolizes extracellular.[14] Taking all these considerations into account, the *in vivo* protocol was developed based on the induction of contact dermatitis in rats using a dispersion of nickel sulfate in solid Vaseline at 5%, carrageenan induce inflammation and their irritation study. Inflammation is the one of the most important clinical symptom associated with psoriasis and dermatitis so, it was also investigated in this studies. High amount of surfactant and co-surfactant was used in nanoemulsion, which has irritation potential so, irritation study was also performed.

2. Materials and methods

2.1. Materials

Clobetasol propionate was purchased from Mahima Lifesciences Pvt. Ltd. (New Delhi, India). Eucalyptus oil was obtained from Scientific International (New Delhi, India). PEG 200, Tween 80, Tween 20, Pleural oleic, Glycol, Brij35, Propanol, Isopropyl alcohol and Ethanol were purchased from Merck (Merck, India). Labrasol, Safsol and Capryol were obtained as a kind gift from Gattefosse (Mumbai, India). All other chemicals used were of analytical grade.

2.2. Preparation of optimized nanoemulsion

On the basis of solubility studies Eucalyptus oil, Tween 20 and ethanol were selected as oil, surfactant and co-surfactant respectively. Different combination of surfactant and co-surfactant were prepared commonly known as S_{mix}. To find out the nanoemulsion region pseudo ternary phase diagrams were constructed for each S_{mix} ratio. Finally S_{mix} (1:2) was selected for the preparation of nanoemulsion. A number of nanoemulsion formulations were prepared based on the different ratio of oil: S_{mix}(1:2): water and evaluated for their physical stability studies. Considering the irritation potential of surfactants, S_{mix} ratio containing minimum percentage of surfactant was selected for the preparation nanoemulsion. Optimized nanoemulsion was prepared by dissolving 0.05% (w/v) of CP in 10% (v/v) eucalyptus oil then 35% (v/v) mixture of Tween-20 and ethyl alcohol (1:2 v/v) were added slowly in oil phase. Then remaining amount of distilled water was added slowly to get the final preparation of 100% (v/v).[15]

2.3. Animal ethics

Either sex Wistar rats were separated into groups. These animals, weighing 180–200 g, were maintained in a standard dark–light cycle at room temperature. The rats had free access to food (standard laboratory rat chow) and water. The protocol to carry out *in vivo* studies was approved by the animal ethics committee, SBS College of Pharmacy, Patti, Amritsar, Punjab; India. The committee's guidelines were followed for the studies.

2.4. Dermatitis induction by 5% nickel sulfate

Contact dermatitis was induced by 5% nickel sulfate in solid Vaseline similar to the procedure adopted by Fontana et al[16,17] Animals were divided into five sets ($n = 8$). After tricotomization, all groups received sensitization with nickel sulfate in the abdomen, except the first group which received only solid Vaseline and continued under the same environmental and feeding conditions as the other groups, this being the control group (C). The induction of dermatitis was done 6 days after sensitization by nickel sulfate in solid Vaseline (5 applications with an interval of 72 h) in each ear after tricotomization. The first group which received only solid Vaseline was euthanized 72 h after the last application of the sensitization agent. The second group was induced to allergic contact dermatitis, which was not managed, and the rats were euthanized 72 h after the last application of nickel sulfate, being the positive control (D). The third group received the topical administration in each ear of the placebo nanoemulsion the fourth received topical administration in each ear of the clobetasol-loaded nanoemulsion and fifth group received marketed cream (Glevate cream). Dose of application for nanoemulsion and marketed formulation was equal to 1 μg of clobetasol propionate. Group 4 & 5 were treated daily with 0.5 ml of the drug loaded nanoemulsion and marketed cream (Glevate cream) for 5 days on days 1, 3, and 5. All formulations were applied uniformly throughout the ear tissue with massage in order to obtain a better drug penetration. After the completion of each treatment, the animals were euthanized and the blood