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Research paper

Lecithin vesicles for topical delivery of diclofenac

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Abstract

Skin penetration of topically applied diclofenac is important for the treatment of rheumatic diseases and actinic keratoses. We have studied the permeation of diclofenac across human cadaver epidermis in-vitro from four lecithin vesicle formulations and a few marketed semi-solid preparations. The lecithin vesicle formulations were prepared by dissolving the lipid contents (lecithin and sodium cholate) in a 1:1 mixture of methanol-chloroform, evaporating the solvents under vacuum, and hydrating the lipid layer with the drug solution in water or 10% ethanol. The vesicles were sonicated for 5 min to reduce the vesicle size and their size and Zeta potential were characterized. The cumulative amount and maximum flux of diclofenac was 69.7 ± 40.3 μg and 4.77 ± 3.16 μg/h cm^2 from lecithin vesicles containing sodium cholate and 10% ethanol, and is the highest of all formulations studied. The cumulative amount and mean maximum flux obtained from other formulations were in the range of 2.46 ± 1.98–29.9 ± 10.1 μg and 0.53 ± 0.46–3.61 ± 0.86 μg/h cm^2. Based on the results, lecithin vesicles of diclofenac appear to be advantageous for the topical delivery of diclofenac.

Keywords: Topical diclofenac; Lecithin vesicle; Rheumatic disease; Actinic keratosis

1. Introduction

Diclofenac is one of the routinely prescribed anti-inflammatory agents available for the management of pain and inflammation [1]. It is marketed as injections, oral sustained release tablets and topical formulations. The drug is almost completely absorbed after oral administration but is subjected to ~50% hepatic first-pass metabolism. Owing to its short biological half-life, a sustained action dosage form is generally preferred. Although a major portion of commercial diclofenac is available in the form of oral medications, the drug causes serious adverse effects in the gastrointestinal tract. Gastrointestinal bleeding and ulcerations are quite common due to oral diclofenac [2]. Clinical evidence suggests that topically applied non-steroidal anti-inflammatory drugs (NSAIDs) are safer and at least as efficacious as oral NSAIDs in the treatment of rheumatic diseases [3]. Gastrointestinal adverse drug reactions after topical NSAID use are rare when compared to the 15% incidence of serious GI events associated with oral NSAIDs. However, formulation may have a dramatic impact on depth of penetration at the site of application, retention of drug molecules within the layers of skin, concentrations achieved in the muscle tissue, synovial fluid and in systemic circulation. Irrespective of the target tissue, whether it is epidermis for treating actinic keratoses (AK) or the muscle and synovial fluid in rheumatic disease, a topically applied NSAID has to cross the natural barrier, stratum corneum of skin. The purpose of the present study was to evaluate the ability of lecithin vesicles to facilitate penetration of diclofenac through stratum corneum barrier and release behavior of the drug across cadaver epidermis in vitro. Further, topical preparations of diclofenac have already been available in Asian and European markets for a few years and recently, the United States Food and Drug Administration has approved the marketing of Solaraze® (diclofenac cream) in the USA. Thus, a comparative account of the ability of lecithin vesicles and different topical formulations available in the market to release diclofenac across the epidermis should provide information regarding

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their potential in treating rheumatic diseases. The ability of lecithin vesicles to deliver diclofenac has been recently demonstrated in animal models [4].

2. Materials and methods

2.1. Materials

Soya phosphatidylcholine, sodium cholate, diclofenac, ethyl alcohol and chloroform were purchased from Sigma Scientific and Methanol from Fischer Scientific. Diclofenac topical formulations Dicloran® gel (Lekar Pharma Ltd., Daman, India), Omnigel® (Cipla Ltd., Mumbai, India), Diclotal® gel (Blue Cross Laboratories Ltd., Verna, Goa, India), Voveran® Emulgel (Novartis, India), Jonac® gel (German Remedies Ltd., Mumbai, India) are all marketed in India and were obtained from a pharmacy. All these preparations contained diclofenac equivalent to 1% w/w of diclofenac sodium and were manufactured within 3 months. Human cadaver skin was obtained from the skin bank of the Department of Dermatology, Detroit Medical Center, Detroit, MI and stored at −80 °C until used.

2.2. Methods

2.2.1. Separation of human epidermis

The epidermis was separated from full thickness human skin (from a single donor) that was obtained from skin bank and frozen until use. Separation of epidermis at the epidermal/dermal junction was achieved by immersing the full thickness skin in water at 60 °C for 2 min and gently peeling the epidermis. The physical integrity of the epidermal sheets was assessed by visual examination. The integrity of barrier function in the heat-separated epidermis was tested by measuring trans-epidermal resistance.

2.2.2. Preparation of lecithin vesicles of diclofenac

Four lecithin vesicle formulations of diclofenac with compositions shown in Table 1 were prepared in the laboratory. Soya phosphatidylcholine alone or along with sodium cholate was dissolved in a 1:1 mixture of methanol and chloroform and the solution was dried using a rotary evaporator. The film was hydrated with the diclofenac solution and the preparation was sonicated for 5 min in a bath sonicator to reduce the size of the vesicles. Size and Zeta potential (ZP) of the vesicles were determined using a Zetasizer 3000HS (Malvern instruments, UK) with an angle of detection of 90°. Distribution of diclofenac in the vesicles was tested by assaying the drug in aliquots (triplicate) and was found to be greater than 97 ± 5.2% of the labeled amount.

Table 1
Composition of lecithin vesicles of diclofenac

Ingredient	Formula			
	F1	F2	F3	F4
Diclofenac sodium	100 mg	100 mg	100 mg	100 mg
Soya phosphatidylcholine	440 mg	440 mg	440 mg	440 mg
Sodium cholate	–	–	150 mg	150 mg
Ethyl alcohol	–	1 ml	–	1 ml
Distilled water	10 ml	9 ml	10 ml	9 ml

2.2.3. Permeation/flux studies

Modified Franz cell with a diffusion area of 1 cm^2 and a receiver volume of 7.5 ml were used in passive diffusion studies and all experiments were conducted in quadruplicate. Phosphate buffer of pH 7.4 was used as the receiver medium. A suitable size of heat separated human epidermis was die cut and mounted in the Franz cell, with the stratum corneum side facing upward. After filling the donor compartment with the diclofenac formulation (1.5 ml in the case of formulations F1–F4 and diclofenac equivalent quantities in the case of marketed preparations) and the receiver compartment with receiver fluid (phosphate buffer, pH 7.4), the receiver compartment was connected to a continuous-flow peristaltic pump with a reservoir on one side and a sample collector on the other side. Samples were collected using a fraction collector that collects 1.5 ml every 90 min. Aliquots of the collected samples were analyzed for diclofenac content using a modified high-performance liquid chromatography (HPLC) method reported.

2.2.4. HPLC method of analysis

The diclofenac assay was based on reported literature and slightly modified to avoid any interference by solvents [5]. Briefly, the method employed a reverse-phase HPLC (Hewlett Packard) with a 4.6 × 250 mm All-Tech C-18 column. A mobile phase consisting of 60% acetonitrile and 40% of 0.5% acetic acid was used. A flow rate of 2 ml/min was used and diclofenac was monitored by UV detection at 280 nm. The retention time of diclofenac was 3.2 min. The area under the peak was used to calculate the concentration of diclofenac using external standards that showed a linearity over the concentration range of 0.03 to 1 μg/ml. The intraday and interday variations of the method were less than 2%.

2.2.5. Flux calculations and statistics

Amount of diclofenac released in successive 1.5-h intervals was obtained from the cumulative amount released at different time intervals and the flux was calculated per unit area and time. Cumulative amount of diclofenac released at the end of 18 h and the maximum flux values for different formulations were compared using analysis of variance and multiple comparison procedures (the Tukey test) employing SigmaStat v.2.03 at $P < 0.01$. Lag times were obtained by extrapolating the cumulative amount of diclofenac released versus time curves to the time axis.

3. Results

Mean size and Zeta potential values of lecithin vesicles of diclofenac are given in Table 2. Inclusion of 10% alcohol in the preparation has lowered both size and ZP of the lecithin vesicles. Plots of mean cumulative amount of diclofenac released across cadaver epidermis from different formulations evaluated as a function of time are shown in Fig. 1. The lag times for the detection of diclofenac in the receiver compartment for different formulations tested are shown in Table 3. The mean lag time in the case of formulations containing cholate (F3 and F4) was much shorter than all other formulations tested. The cumulative amount of diclofenac released from different formulations is given in Table 3. Comparison of the mean values using analysis of variance has revealed that they are significantly different ($P < 0.001$). Further, pairwise multiple comparison procedures (the Tukey test) have revealed that the drug released from formulation F4 was significantly higher ($P < 0.01$) than from all other formulations tested. The plots of mean trans-epidermal flux of diclofenac versus time shown in Fig. 2 also revealed that formulation F4 containing sodium cholate and 10% alcohol exhibited maximum flux. The trans-epidermal maximum flux values for diclofenac from different formulations are given in Table 3. Almost all the formulations reached 75–90% of maximum flux in about 6–9 h. However, pairwise multiple comparison procedures (Tukey's test) revealed that the maximum flux of F4 is significantly higher ($P < 0.05$) than those of F1, F3, Dicloran® and Diclotal®, but not significantly different from those of F2, Omnigel®, Voveran® and Jonac®.

4. Discussion

NSAIDs are routinely prescribed for patients with rheumatic disease and such patients are at increased risk for serious gastrointestinal complications, when administered by the oral route. Topical administration of NSAIDs offers the advantage of local, enhanced drug delivery to affected tissues with reduced bioavailability (5–15%) as well as incidence of systemic adverse effects, such as peptic ulcer disease and gastrointestinal hemorrhage. Thus, a well-formulated topical preparation that can effectively deliver diclofenac to the site of action in rheumatic diseases and/or AK will go a long way in reducing the gastrointestinal side effects of the drug. In this study, we have evaluated the in vitro release of diclofenac across human cadaver epidermis using lecithin vesicles of the drug and compared the release rate with those of some topical diclofenac formulations marketed in India. Based on the cumulative amount of diclofenac released at 18 h and the maximum flux values, the formulations evaluated in this study in the decreasing order of their ability to deliver the diclofenac across epidermis is formulation F4 > Jonac® > Omnigel® > Voveran® > Diclotal® > F2 > F3 > F1 > Dicloran®. It is important to note the effect of alcohol in the formulation on the size and ZP of vesicles and the epidermal flux of diclofenac. Addition of 10% alcohol to formulation F1 resulted in smaller vesicles with lower ZP (F2, Table 2) and higher flux as well as amount of drug released (Table 3). Further, although the base composition of the marketed formulations is not known, the product that is stated to contain > 50% alcohol in base (Jonac®) ranked second amongst all the formulations, thus reconfirming the ability of alcohol to improve the permeability of the stratum corneum. Inclusion of both alcohol and sodium cholate in F1 further reduced both size and ZP of vesicles, and resulted in a significant decrease in lag time and increase in CAR and the maximum flux of diclofenac severalfold (F4, Table 3). The upper layers of the skin are known to have an isoelectric point of pH 4, so the pores in the stratum corneum will have a negative charge in a solution with pH 4 or higher [6]. Thus,

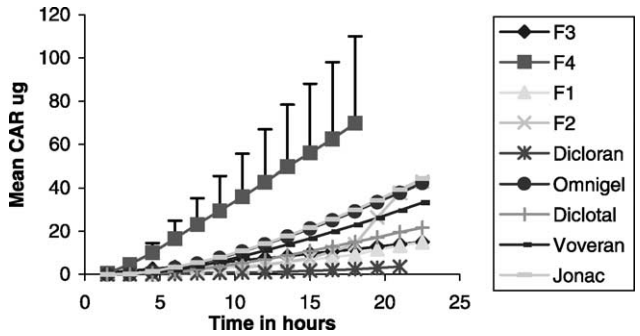

Fig. 1. Mean cumulative amount of diclofenac released (μg) across cadaver epidermis by passive diffusion in vitro from lecithin vesicles (F1–F4) and marketed formulations of the drug.

Table 2
Size and Zeta potential (ZP) of lecithin vesicles of diclofenac

Preparation	Size, mean ± SD (nm)	ZP, mean ± SD (−mV)
F1	125.8 ± 0.8	70.5 ± 0.5
F2	86.7 ± 0.5	61.8 ± 2.1
F3	132.0 ± 0.8	71.6 ± 0.2
F4	77.2 ± 0.2	52.8 ± 0.1

Table 3
Lag time, cumulative amount of diclofenac released (CAR) at 18 h and the maximum epidermal flux values for different diclofenac topical preparations

Formulation	Lag time, mean ± SD (h)	CAR of diclofenac, mean ± SD (μg)	Flux of diclofenac, mean ± SD (μg/h cm^2)
F1	4.63 ± 0.83	9.20 ± 1.7	1.12 ± 0.27
F2	3.63 ± 2.14	14.00 ± 7	2.33 ± 2.16
F3	1.00 ± 0.43	11.70 ± 5.8	0.75 ± 0.25
F4	1.28 ± 0.1	69.70 ± 40.3	4.77 ± 3.16
Dicloran	3.23 ± 3.67	2.46 ± 1.98	0.53 ± 0.46
Omnigel	3.42 ± 0.65	29.20 ± 3.2	2.99 ± 0.52
Diclotal	3.45 ± 0.81	14.95 ± 3.2	1.57 ± 0.23
Voveran	3.64 ± 0.73	22.90 ± 4.7	2.36 ± 0.32
Jonac	3.15 ± 0.78	29.90 ± 10.1	3.61 ± 0.86

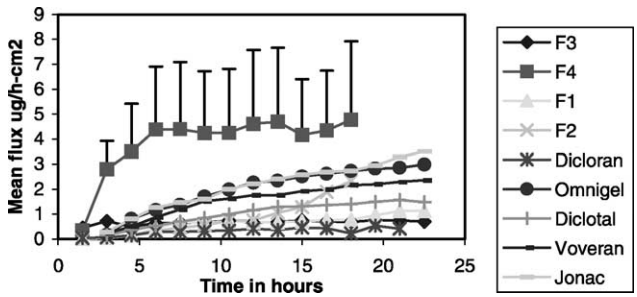

Fig. 2. Mean trans-epidermal passive flux of diclofenac in vitro from lecithin vesicles (F1–F4) and marketed formulations of the drug.

the lower ZP in combination with smaller size of vesicles of F4 might have facilitated their penetration through the stratum corneum. Further, addition of only sodium cholate to F1 did not result in any appreciable change in size as well as ZP of vesicles (F3). However, sodium cholate inclusion appears to have imparted flexibility to vesicles resulting in almost similar lag times for formulations F3 and F4. According to Cevc et al. [4,7], the presence of sodium cholate imparts flexibility to the bi-layer lipid membranes of lecithin vesicles, thus enabling them to pass through pores many-fold smaller than the vesicle size spontaneously under the influence of trans-epidermal water activity gradient. The ability of such flexible vesicles (transfersomes) to passively transport large molecular weight proteins/peptides has been reported [8,9]. Also, Cevc and Blume have demonstrated that therapeutically meaningful diclofenac concentrations can be achieved in the target tissues of mice, rats and pigs using lipid vesicles [4]. Thus, the results of the present study along with those of Cevc and Blume advocate the use of lecithin vesicles as drug carriers for delivering diclofenac upon topical application in treating rheumatic diseases, to avoid the gastrointestinal toxicity of oral diclofenac.

Restricting diclofenac delivery to the epidermis is important in the treatment of pre-cancerous lesions like AK. Although we have not analyzed diclofenac content in epidermis at the end of experiments, such an attempt would have revealed the potential use of the preparations tested in the present study to treat AK.

5. Conclusions

This study demonstrated that significant amounts of diclofenac can permeate across the skin barrier when applied as lipid vesicles. The results advocate the need to test the lecithin vesicle formulation F4 to treat rheumatic disorders where facilitated penetration of the drug into muscle and synovial fluid is desirable. Further, the preparation F4 as such or after suitable modifications to confine diclofenac delivery to the epidermis has to be tested for the local treatment of AK.

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