ORIGINAL ARTICLE

Effect of permeation enhancers on the penetration mechanism of transfersomal gel of ketoconazole

Reshmy Rajan, Deepa T. Vasudevan

Department of Pharmaceutics, Amrita School of Pharmacy, AIMS Health Care Campus, Kochi, India

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ABSTRACT

The aim of the present research work was to investigate the potential of transfersome formulations for transdermal delivery of Ketoconazole (KTZ). KTZ is a broad-spectrum antifungal agent that is active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. The transfersomes were formulated by lipid film hydration technique using Rotary vacuum Evaporator. The prepared transfersomes were converted into suitable gel formulation and is evaluated for their gel characteristics like pH, viscosity, spreadability, extrudability, homogeneity, drug content, etc. Suitable essential oils acting as natural permeation enhancers were added to the transfersomal formulation of KTZ for their release studies. Studies proved that addition of suitable permeation enhancers to the transfersomal formulation improved the release and permeation of KTZ, which showed that the permeation enhancers modify the barrier to penetration present in skin without itself undergoing any change. From the various essential oils which are used as permeation enhancers, the formulation containing Eucalyptus oil showed better *in vitro* release and permeation as compared with other formulations containing different permeation enhancers.

Key words: Edge activator, ketoconazole, Lecithin, permeation enhancers, transfersomes

INTRODUCTION

Ketoconazole (KTZ) is a broad-spectrum antifungal agent that is active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. It is effective against yeasts and dermatophytes, both systemically and topically. It is now mainly used systemically in treating life-threatening fungal infections.[1,2] A new vesicular derivative "Transfersomes" has paved the way to minimize the defective transdermal permeation of a number of low and high molecular weight drugs which has been found to be one of the major advancement in vesicle research. A transfersome carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis and thus suitable for controlled and potentially targeted drug delivery. Transfersome is highly adaptable, stress responsive, complex aggregate. Its preferred form is an ultra deformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. This enables the transfersomes to cross various transport barriers efficiently and then act as a drug carrier for noninvasive targeted drug delivery and sustained release of therapeutic agents.[3]

Agents capable of modifying the barrier to penetration presented by the skin are called as Penetration Enhancers or they are the substances which reversibly reduce the barrier resistance of the stratum corneum (SC) without damaging the viable cells.[4] The present work focused on the formulation of KTZ transfersomes and the effect of natural permeation enhancers such as Eucalyptus oil, Turpentine oil, and Peppermint oil on the prepared KTZ transfersomes. Developments in the transdermal delivery of drugs offer a potential solution to improvement in the penetration of antifungal agents into the skin. One such approach has been the application of essential oils, such as eucalyptus oil, Peppermint oil, and Turpentine oil.[5] Eucalyptus oil

Address for correspondence:
Dr. Deepa T Vasudevan,
Department of Pharmaceutics, Amrita School of Pharmacy,
AIMS Health Care Campus, Kochi, India.
E-mail:deepatv@aims.amrita.edu

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is an effective skin penetration enhancer and it contains 1,8-cineole, a monoterpene cyclic ether which can enhance penetration of both lipophilic and hydrophilic compounds.[6-9] Turpentine oil and Peppermint oil also acts as a penetration enhancer. They increase the penetration of drugs when applied on the skin to give a faster onset of action. Here, an attempt has been made to incorporate both the mechanisms of transfersomes and permeation enhancers.

MATERIALS AND METHODS

Materials

KTZ was procured as a gift sample from Aarti Drugs Ltd, Mumbai, and from Chethana Pharmaceuticals, Kerala; Tween 80 from LobaChem Pvt. Ltd, Mumbai; Lecithin from Himedia laboratories Pvt Ltd, Mumbai; and Dichloromethane from Nice Chemicals Pvt Ltd, Kerala. All other ingredients used were of analytical grade.

Methods

Preparation of ketoconazole transfersomes

The transfersomes were prepared in Rotary vacuum evaporator, using lipid film hydration technique. Drug (KTZ), Lecithin (PC), and Edge Activator (Tween 80) were dissolved in dichloromethane which is the solvent. Organic solvent was removed by evaporation at a temperature (40°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15minutes at corresponding temperature.[10,11]

Preparation of topical hydrogel

As a vehicle for incorporation of transfersomes for topical delivery, carbopol hydrogels were prepared. KTZ transfersomes lipid dispersion was utilized for the formulation of topical hydrogel. The transfersome dispersion equivalent to 100 mg of pure drug was taken.[12] Formulations are named T1-T4 and T1 is taken as the control, without adding any permeation enhancers. Eucalyptus oil, Turpentine oil, and Peppermint oil were added to the formulation (15% of drug amount in prepared gel) to the remaining formulations (T2, T3, T4). The various compositions of Lecithin vesicles in the formulations are included in Table 1.

Evaluation of transfersomal gel

Physicochemical evaluation of gels

pH

Direct measurements were made using a digital pH meter (MK-IV SYSTRONICS).

Viscosity determination

Viscosities were determined using cone and plate viscometer (Digital Rheometer model DV1, Brookfield) of the gels prepared. A spindle (no. 7) was rotated at 10 rpm.

Table 1: Composition of Lecithin vesicles

Composition	T1	T2	T3	T4
Drug	100 mg	100 mg	100 mg	100 mg
Lecithin	100 mg	100 mg	100 mg	100 mg
Tween 80	3 drops	3 drops	3 drops	3 drops
Dichloromethane	5 ml	5 ml	5 ml	5 ml
Eucalyptus oil (%)	-	15%	-	-
Peppermint oil (%)	-	-	15%	-
Turpentine oil (%)	-	-	-	15%

Spreadability

Spreadability was determined by applying weight to glass slides into which formulation was placed, and time in seconds required to separate the slides was noted. Spreadability of each formulation was reported in seconds.[13] Spreadability was then calculated by using the formula:

$$S = M.L/T \quad (1)$$

Where, S = spreadability, M = weight tide to upper slide, L = length of glass slide, and T = time taken to separate the slide completely from each other.

Extrudability

Extrudability was measured using a closed collapsible tube containing formulation which was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 seconds was determined.[14] The average extrusion pressure in grams was reported.

Homogeneity test

The formulations were tested for their homogeneity by visual appearance after the gels have been set in the container. Also, a small quantity of each gel is pressed between the thumb and the index finger, and the consistency of the gel is noticed whether homogeneous or not.

Drug content analysis

For the estimation of the drug in gels, KTZ was extracted from 1 g of each gel formulation with 50 ml of methanol for 30minutes, and the resultant mixture was filtered through membrane filter (pore size 0.45 μm). From this, 2.5 ml was pipette out and made up to 10 ml. Then, 1–10 ml, the absorbance of the sample was determined spectrophotometrically at 222 nm. The concentration of KTZ was estimated from the regression equation of calibration curve.[14]

In vitro Drug Release Studies

The *in vitro* drug release from gel formulations was studied across cellulose membranes (Sigma Aldrich) using Franz-type diffusion cells (Orchid Scientifics-FDC-06) with effective diffusional surface area of 3.14 cm^2. The cellulose acetate membrane (cellophane membrane) having a pore size 33 mm was mounted between the donor and receptor compartment of the diffusion cell.[15,16] The receiver compartment was

filled with 15 ml of phosphate buffer pH 7.4 to ensure sink condition. The donor compartment of the cell was filled with 1 g vehicle containing the test drug. 0.5 ml sample was withdrawn at intervals of 1 hour for a period of 24 hours, and each time equal volume was replaced with drug-free receptor fluid.[17,18] All samples were analyzed by UV spectrophotometer at 222 nm. The experiment was carried out in triplicate, and the mean cumulative percentage releases from three batches were calculated.

Ex vivo Study

Franz diffusion cell (with effective diffusion area 3.14 cm^2 and 15 ml cell volume) was used for the drug permeation studies. Phosphate buffer of pH 7.4 was used as the receiver medium. A suitable size of pig skin was cut and mounted in the Franz cell, with the SC side facing upward. Transfersomal gel was applied onto the surface of pig skin evenly. The skin was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS (pH 7.4) solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (0.5 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 222 nm after appropriate dilutions. The cumulative amount of drug released across the skin was determined as a function of time.[17,18]

RESULTS

The results for pH, viscosity, drug content, extrudability, and spreadability of all the prepared Transfersomal gel formulations are shown in Table 2. The pH values of all formulations were found to be acceptable and similar to skin pH, as the formulation is topical. Viscosity is an important parameter for characterizing the gels as it affects the extrudability and release of drug. Viscosity of T1, T2, T3, and T4 were found to be 12000, 11000, 13500, and 12500 cps, respectively. All formulations exhibited good spreadability and extrudability. The value of spreadability indicates that the gel is easily spreadable by small amount by shear. All gel preparations indicate a good spreadability. The extrusion of the gel from the tube is important during its application and in patient acceptance. The extrudability of all formulations was found to be good and compatible. Homogeneity of the various formulations was tested by visual observation and also by applying pressure between the thumb and index finger and was found to be excellent. The drug content in transfersomal gel was found to be 96.5%, 95.1%, 94.3%, 92.2%, respectively. The percentage drug content of all formulations was found to be satisfactory. Hence, methods adopted for gel formulations were found to be suitable (The results are included in Table 2).

The *in vitro* release profile of KTZ from its various transfersomal gel formulations were determined, which showed that the drug release from formulation containing penetration enhancers were higher than its release from control [Table 3]. The drug release from the gels containing penetration enhancers can be ranked in the increasing order as per their respective penetration enhancers as control < peppermint oil <turpentine oil< eucalyptus oil. The results indicate that formulation containing eucalyptus oil has the highest percentage of drug release. The most effective oil was found to be Eucalyptus oil followed by Turpentine oil and Peppermint oil. Eucalyptus oil has shown significant increase in diffusion rate compared with formulations containing Peppermint oil, Turpentine oil, and control which showed minimal release. Eucalyptus oil is an effective skin penetration enhancer and it contains 1,8-cineole, a monoterpene cyclic ether which can enhance penetration

Table 2: Evaluation of transfersomal gel

Formulations	pH	Viscosity (Centi poise)	Spreadability	Extrudability	Drug content (%)	Homogeneity
T1	6.8	12000	++	++	96.5%	++
T2	6.7	11000	++	+++	95.1%	++
T3	6.9	13500	++	++	94.3%	++
T4	6.6	12500	++	++	92.2%	++

(Key + + = Excellent)

Table 3: *In vitro* drug release of various formulations

Time in hrs	T1	T2	T3	T4
1	4.31±0.50	13.22±0.33	7.36±0.39	16.52±0.55
2	5.20±0.21	17.14±0.17	11.56±0.41	22.95±0.5
3	6.19±0.31	22.16±0.75	12.66±0.24	19.22±0.16
4	7.63±0.83	25.32±0.38	16.51±0.62	29.08±0.58
5	8.88±0.85	28.00±0.19	17.69±0.33	31.76±0.11
6	10.43±0.60	30.71±0.45	18.28±0.21	33.44±0.49
7	11.46±0.29	33.71±0.15	19.60±0.32	34.57±0.45
8	13.97±0.20	36.27±0.41	24.61±0.33	37.04±0.39
24	16.24±0.54	44.98±0.35	30.59±0.74	37.55±0.23

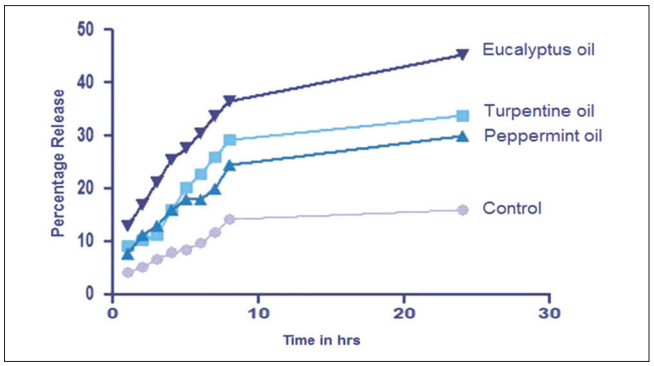

Figure 1: Effect of essential oils on drug release

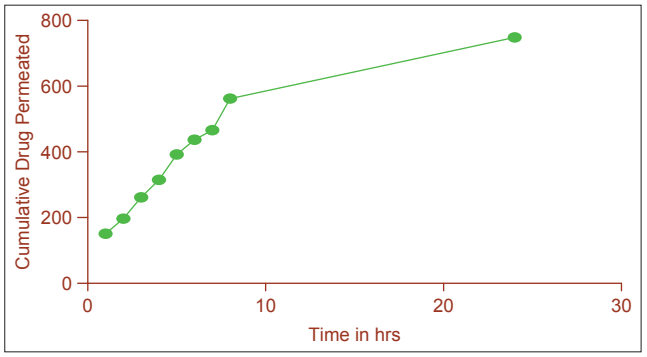

Figure 2: Effect of essential oils on skin permeation study

of both lipophilic and hydrophilic compounds. Terpenes, including 1,8-cineole, bind to the SC and are thought to enhance lipophilic drug penetration by increasing the partition coefficient and hydrophilic drug penetration by increasing the diffusion coefficient. 1,8-cineole has been found to increase skin penetration by disrupting intercellular lipids in SC and to change SC membrane fluidity at the concentrations as low as 1% to 5% [Figure 1].

The formulation showing better release was used for skin permeation studies (T2), which showed a better skin permeation activity too [Figure 2 and Table 4].

DISCUSSIONS

KTZ is a broad-spectrum antifungal agent active that is against a wide variety of fungi and yeasts. The special drug carrier Transfersomes help in the noninvasive delivery of pharmaceuticals across the skin. KTZ from these ultra deformable vesicles can penetrate more deep into the soft tissue in presence of the penetration enhancers. Thus, the transfersomal gel of KTZ combines the safety, efficacy, and penetration activity. From the different penetration enhancers used, the results concluded that Transfersomal formulations containing Eucalyptus oil are better for penetration activity. Eucalyptus oil contains 1,8-cineole as its main constituent and a series of 17-monoterpene and terpenoids that have proven penetration enhancement

Table 4: *Ex vivo* data of formulation (T2)

Time in hrs	Trial 1	Trial 2	Trial 3	Mean	Standard deviation
1	150.88	150.45	149.07	150.13	0.942
2	196.80	194.76	195.34	195.63	1.046
3	314.92	313.86	313.89	314.22	0.605
4	261.89	260.97	260.89	261.25	0.551
5	292.57	291.98	290.34	291.63	1.151
6	436.96	435.89	436.90	436.58	0.602
7	466.44	466.34	465.98	466.25	0.243
8	562.04	563.00	563.09	562.71	0.581
24	748.22	749.08	748.01	748.44	0.567

effect. Thus, we can utilize the combined effect of both transfersomes and penetration enhancers for the topical delivery of the antifungal drug KTZ.

CONCLUSIONS

Novel vesicular drug delivery system has been used nowadays for the therapeutic effectiveness of topically applied drugs. In the present study attempts were made to formulate and evaluate transfersomal gel of ketoconazole. All the gels were evaluated for their pH, drug content, viscosity, spreadability, extrudability and *in vitro* release (using cellophane membrane and pig ear skin). The pH range of the formulated gels was found to be suitable for topical application and the drug content was found in the range of 95.08-97.65%. The viscosity, spreadability and extrudability were evaluated. The *in vitro* drug release was carried out using cellophane membrane and pig ear skin in phosphate buffer pH 7.4. Among all the formulations the drug release was greater in formulation containing eucalyptus oil compared to other penetration enhancers and control. Among all the penetration enhancers used eucalyptus oil shows better penetration than peppermint oil, turpentine oil and control.

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