Research Article

ISSN	Print	2231 – 3648
	Online	2231 – 3656

International Journal of Pharmacy and Industrial Research

Available Online at: www.ijpir.com

TRANSDERMAL DELIVERY OF KETOCONAZOLE VIA LIPOSOMAL CARRIER SYSTEM

[*1]Shaik. Harun Rasheed, [2]Padmabhushanam

[1]Department of Pharmaceutics, BA&KR College of Pharmacy,
Ongole-523001, Andhra Pradesh, India.

[2]Department of Pharmaceutical Analysis, Monad University, Hapur, U.P, India.

Abstract

Antifungal drug, Ketoconazole was encapsulated in liposomes for topical application. The main objective of the present work was to formulate and characterized a liposomal carrier containing ketoconazole and to investigate the drug delivery of ketoconazole which is an antifungal drug having limited transdermal permeation. The liposomes were characterized for size, entrapment efficiency, skin penetration, vesicle skin interaction and stability study. The partical size was determined by photo correlation spectroscopy which was found to be 222±15nmfor empty vesicle and 264±12nmfor drug loaded vesicle, encapsulation efficiency was determined by dialysis technique (40.12±1.9%).Skin penetration can be determined by using confocal laser scanning microscopy (CLSM) (168µm). Vesicle skin interaction study can be determined by using optical microscope, stability of ketoconazole loaded liposomes can be determined by assessing the size and structure of vesicle over time. Our results indicate that ketoconazole loaded liposomal carrier could be better choice for the treatment of number of dermal infections.

Key words: Liposome, Ketoconazole, vesicle, Confocal laser scanning microscopy, Skin penetration.

Introduction

Human skin is an effective, selective barrier to chemical permeation, although the skin as a route for delivery can offer many advantages, including avoidance of first-pas metabolism, lower fluctuations in plasma drug levels, targeting of the active ingredient for a local effect and good patient compliance.[1] Water soluble molecules and drugs are normally not able to cross the skin as the skin is a natural barrier to water. The stratum corneum is composed of insoluble bundled keratins surrounded by a cell envelope, stabilized by cross-linked proteins and covalently bound lipids. In general, the epidermis (specifically the stratum corneum) provides the major control element; most small, water-soluble, and non-electrolytes diffuse into the systemic circulation a thousand times more rapidly when the horny layer is present.[2] Thus, to maximize the flux of the drug, the barrier hindrance is reduced by various approaches. Several technological advances have been made in the recent decades to overcome skin barrier properties. Examples include physical

Author for Correspondence:
Shaik.Harun Rasheed,
Department of Pharmaceutics,
BA&KR College of pharmacy, Doddavarapadu,
Ongole-523001, Andhra Pradesh, India.
Email ID: shaikharunrasheed@gmail.com

means such as iontophoresis, sonophoresis, microneedles, and chemical means, using penetration enhancers and biochemical means, such as, liposomal vesicles and enzyme inhibition. The physical means like iontophoresis, microneedles, and sonophoresis are relatively complicated to use, and will affect patient compliance.[3] The use of chemical enhancers such as surfactants and organic solvents induce irritation, cause damage, and reduce skin barrier function, therefore, it is desirable to deliver the therapeutic agents that maintain the normal skin barrier function without the aid of a chemical enhancer.[4]

One such approach is the use of vesicular systems. In the past decade, topical delivery of drugs by liposomal formulation has evoked considerable interest. Deformable liposomes[5] and transferosomes were the first generation of elastic vesicles introduced by Ceve and Blume, in 1992, and were reported to penetrate intact skin while carrying a therapeutic concentration of drugs, when applied under non occluded conditions.[6] The drug, encapsulated in lipid vesicles, prepared from phospholipids and nonionic surfactants is known to be transported into and across the skin . The lipids present in the skin contribute to the barrier properties of the skin and prevent the systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as nontoxic penetration enhancers for drugs.[7]

In addition, the vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry a significant quantity of drugs across the skin, thus enhancing the systemic absorption of drugs.[8]

Liposomes are microscopic lamellar structures formed on the admixture of soya lecithin, cholesterol and tocopheryl acetate with subsequent hydration in aqueous media. Liposomes have been widely evaluated for controlled and targeted drug delivery for anticancer agents,[9-11] antiparasitic,[12] anti - bacterial,[13] antifungal drugs,[14] antiviral,[15,16] and ocular liposomes.[17-19] Liposomes are found to be suitable for localization of topically applied drugs at or near the site of application, due to fact that they may act as slow releasing vehicles.

Ketoconazole (KTZ) is a broad spectrum antifungal agent active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. Topically it is used in the treatment of candidal or tinea infections of the skin. Encapsulation of Ketoconazole in liposomes may increase the half life providing prolonged drug delivery and minimize the commonly occurring side effects. The current study was aimed to investigate the potential of liposomes in enhancement of Ketoconazole transport across the skin, characteristics of liposomes and their *in-vitro* skin permeation behavior.

Materials and methods

Materials

Ketoconazole was obtained as a gift sample from Medreich pharmaceutical company, Bangalore. Lipoid S phosphatidyl choline-3, containing not less than 98% phosphatidyl choline was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Ethanol and methanol was purchased from Sigma Lab, New Delhi. All other chemicals used in our work were of analytical grade.

Preparation of ketoconazole loaded liposomes

Liposomes containing 2% Lipoid S PC-3 (PC) and Ketoconazole were prepared by the classic mechanical-dispersion method **[20]**. Briefly, a methaniolic solution of Lipoid S PC-3 (PC) was first completely dried in a rotary evaporator (Rotavapor-R, Buchi, Germany) at 550C until a thin lipid film on the wall of a round-bottomed flask was obtained. The resulting lipid film was then hydrated with solution of Ketoconazole(prepared by methanol: water) which was mixed at 700 rpm with a mechanical stirrer (Remi Equipment, Mumbai, India) atroom temperature. For preparation of liposome containing Ketoconazole and D-289, the probe was dissolved together with Lipoid S PC-3 (PC) in the methanol: water mixture.

Determination of liposomes encapsulation efficiency

Liposomes encapsulation efficiency was determined using the dialysis technique for separating the non-entrapped drug from liposomes. Then, according to this method, 3 mL of drug-loaded liposomal dispersion was dropped into a cellulose acetate dialysis bag (Spectra/ Por, MW cut-off 12,000 Spectrum, Canada) immersed in 150 mL of a 50:50 v/v water–methanol solution and magnetically stirred at 30 rpm. Samples were taken at regular time intervals from the receiver, solution were replaced with equal volumes of fresh solvent. Ketoconazole was spectrophotometrically assayed at 222 nm[21, 22] (UV-1700 Shimadzu). The experiment was stopped when constant drug concentration values were obtained in subsequent withdrawals from the receiver phase (taking into account the progressive dilution of the medium). The percent of encapsulation efficiency (EE %) was then calculated according to the following equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug-diffused drug}}{\text{Total drug}} \times 100$$

Each result is the mean of at least three separate experiments.

Determination of liposomal particle size

The average diameter and size distribution of liposome dispersions were determined by photocorrelation spectroscopy using a 90 Plus Particle Size Analyzer (Brookhaven Instrument, New York, USA) at a fixed angle of 90◦ and at 25 ◦C. Liposome dispersions were suitability diluted with phosphate buffer (pH 7.4) and filtered through a 1 µm polycarbonate membrane to minimize interference particulate matter before sizing.[23] Each measurement was in triplicate.

Confocal laser scanning microscopy (CLSM)

CLSM was used to investigate depth and mechanism of skin penetration of ketoconazole loaded liposome preparation, as reported previously. Briefly, unentrapped probe was removed from probe-loaded vesicles by minicolumn ultracentrifugation thereafter formulation was applied non-occlusively for 8 hr to the dorsal skin of 5–6week old nude albino rat. The rat was then sacrificed by heart puncture, dorsal skin was excised, washed, placed on aluminium foil and adhering fat and/or subcutaneous tissue was removed. The skin was then sectioned into the pieces of 1 mm size and evaluated for depth of probe penetration for formulation. The full skin thickness was optically scanned at different increments through the z-axis of a confocal laser scanning microscope (CLSM 510 with an attached universal Zeiss epifluorescence microscope). All investigations were performed as per the protocol approved by the Institutional Animals Ethical Committee (Reg. No. 949/a/06/CPCSEA).[24]

Vesicle skin interaction studies

To observe the ultrastructural changes in the skin upon exposure to liposomal formulation the dispersion was applied on the skin of rats (Male Sprague Dawley, 5–6 week old, 80–100 g). Preparations were applied topically to the skin for 6 h, animals were sacrificed, skin were excised and stored in formalin solution (10%) in phosphate buffer saline (pH 7.4) followed by dehydration with alcohol. It was then treated with anti-media and embedded in paraffin for fixing. Controls skin section was prepared by similar procedure without application of any preparation. Sections of 5 µm thickness were cut from each piece and stained with heamotoxyline and eosin and histological changes in stratum corneum, epidermis and dermis were examined under optical microscope (Leica, DMLB, Heerbrugg, Switzerland).

Results and discussion

Liposomal formulation containing ketoconazole was prepared by using Lipoid S PC-3 (PC), methanol and ketoconazole, photo correlation spectroscopy using a 90 plus particle size analyzer (Brookhaven Instrument, New York, USA) was used to determined the average particle size of the liposomal vesicle and it was found that the size of the vesicle was 222±15nm for empty vesicle and

264±12nm for drug loaded vesicle. In terms of entrapment efficiency, The ketoconazole loaded liposomal formulation showed the good entrapment efficiency (40.2±1.9%) and optimum size (264±12nm) thus showing the good opportunity to the ketoconazole loaded liposomal preparation to attain a better skin penetration, by providing a safe homing to the ketoconazole and optimized vesicular size which has been reported to affect the skin permeation parameters. Confocal laser scanning microscopic studies should be performed to determine the extent of penetration and transdermal potency of the stored system, from the study it showed that a liposomal formulation containing ketoconazole penetrate in the skin up to 168 µm shown in fig. 2. This showed efficient delivery of ketoconazole loaded liposomal vesicle in the skin. This suggests a stable nature of liposomal preparation along with no change in transdermal potency of the stored system.

Fig. No. 01: The application of the liposomes on nude skin of rats

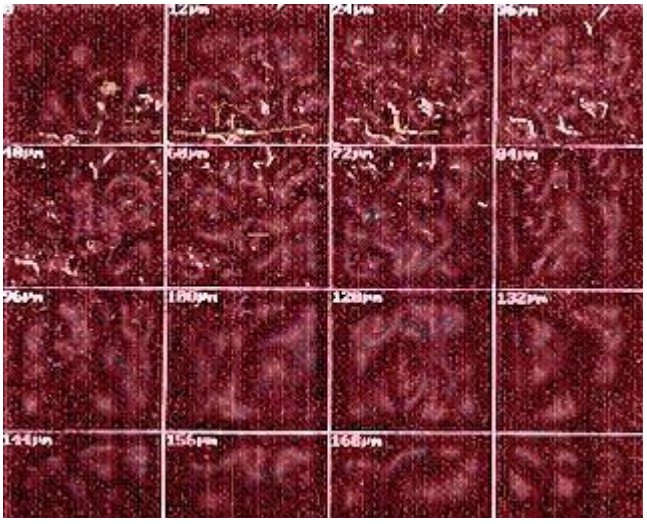

Fig. No. 02. Confocal laser scanning photomicrograph of penetration of ketoconazole from liposomes applied nonocclusively onto nude rat skin.

Liposomes skin interaction studies was performed by skin histopathology on application of liposomal system, it showed that there was no specific changes in the skin histopathology, though skin lipid fluidization could be observed in the form of some penetration pathway, which could be followed by these liposome. Further, mild swelling of corneocytes could also be observed, which suggesting the retention of fluids and thus providing an insight on sustained drug delivery mechanism of ketoconazole loaded liposomal preparation shown in figure 3 (a) and (b). The skin tolerability/irritancy study was performed

to determined whether liposome preparation show any irritation on applying to the skin. As, skin non-irritancy of ketoconazole loaded liposomal formulation was performed by observing the erythema scores upon exposure of hairless rabbit skin to liposome formulation, it was revealed that ketoconazole loaded liposomes showed no significant erythema.

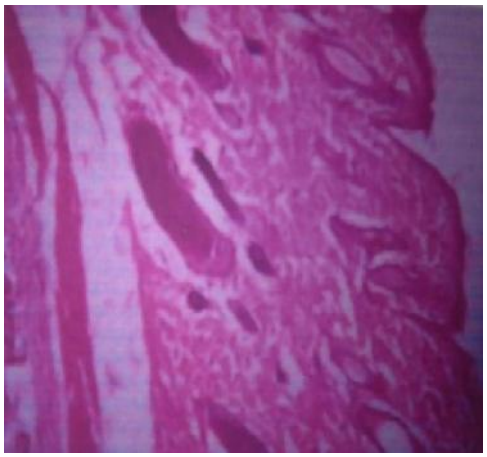

Fig. No. 03 a): Histopathology of the normal rat skin

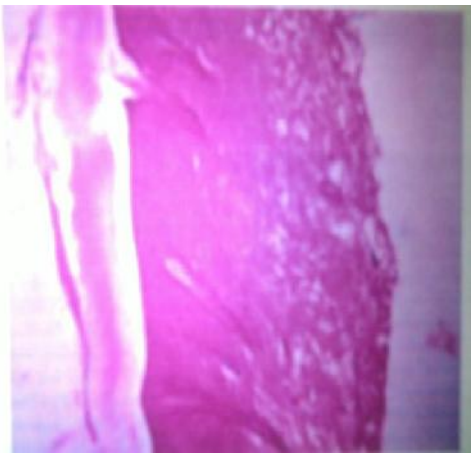

Fig. No. 03 b): Histopathology of the rat skin after application of liposomes

Conclusion

Liposomal formulation of ketoconazole allowed a significant improvement of its therapeutic effectiveness in terms of drug permeation. Also from the study it was confirmed that liposomal formulation of ketoconazole showed a good entrapment efficiency and better stability profile. Thus it concluded that formulation is a very promising carrier for transdermal delivery and creating a new opportunities for topical application of ketoconazole in the fungal infections.

Acknowledgment

The authors are grateful to Medreich pharmaceutical company, Bangalore, for providing ketoconazole to carry out the research work. They are also thankful to Lipoid GmbH (Ludwigshafen, Germany) for providing the gift sample.

References

1. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci., 2001, 14, 101-14.
2. Hadgraft J. Passive enhancement strategies in topical and transdermal drug delivery. Int JPharm,1999, 184, 1-6.
3. Thong HY, Zhai H, Maibach HI. Percutaneous enhancers: An overview. J Skin Pharmacol Physiol, 2007, 2, 1-12.
4. Shim J, Kang HS, Park WS, Han SH. Transdermal delivery of minoxidil with ethanolic liposomes. J Cont Rel., 2003, 97, 477-84.
5. Essa AE, Bonner MC, Barry BW. Electroporation and ultradeformable liposomes. Human skin barrier repair by phospholipids. J Cont Rel., 2003, 92, 163-72.
6. Nguyen PL, Bowstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Disc Tec.. 2005, 2, 67-74.
7. Biju S, Talegoankar S, Mishra P, Khar RK. Vesicular systems An overview. Ind J Pharm Sci., 2006, 5, 141-50.
8. Ceve G. Lipids vesicles and other colloids as drug carrier on the skin. Adv Drug Del Rev., 2004, 56, 675-711.
9. Immordino ML, Brusa P, Rocco F, Arpicco S, Ceruti M, Cattel L. Preparation characterization cytotoxicity and pharmacokinetics of liposomes containing lipophilic gemcitabine prodrugs. J Control Release., 2004, 100, 331-46.
10. Mamot C, Drummond DC, Hong K, Kirpotin DB, Park JW. Liposome-based approaches to overcome anticancer drug resistance. Drug Resist Updat. 2003; 6: 271-9.

11. Jaracz S, Chen J, Kuznetsova LV, Ojima I. Recent advances in tumor-targeting anticancer drug conjugates. Bioorg Med Chem., 2005, 13, 5043-54.
12. Alving CR, Steck EA, Chapman WL Jr, Waits VB, Hendricks LD, Swartz GM Jr, *et al*. Liposomes in leishmaniasis: Therapeutic effects of antimonial drugs, 8-aminoquinolines and tetracycline. Life Sci.,1980, 26, 2231-8.
13. Rukholm G, Mugabe C, Azghani AO, Omri A. Antibacterial activity of liposomal gentamicin against Pseudomonas aeruginosa: A time-kill study. Int J Antimicrob Agents., 2006, 27, 247-52.
14. Chakraborty R, Dasgupta D, Adhya S, Basu MK. Cationic liposome encapsulated antisense oligonucleotide mediates efficient killing of intracellular Leishmania. Biochem. J., 1999, 340, 393-6.
15. De Mareuil J, Mabrouk K, Doria E, Moulard M, de Chasteigner S, Oughideni R, *et al.* Liposomal encapsulation enhances antiviral efficacy of SPC3 against human immunodeficiency virus type-1 infection in human lymphocytes. Antiviral Res., 2002, 54, 175-88.
16. Tadakuma T, Ikewaki N, Yasuda T, Tsutsumi M, Saito S, Saito K. Treatment of experimental salmonellosis in mice with streptomycin entrapped in liposomes. Antimicrob Agents Chemother., 1985, 28, 28-32.
17. Rathod S, Deshpande SG. Albumin microspheres as an ocular delivery system for pilocapine nitrate. Indian J Pharm Sci., 2008, 70, 193-7.
18. Durrani AM, Davies NM, Thomas M, Kellaway IW. Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug delivery system. Int J Pharm., 1992, 88, 409- 15.
19. Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: In vitro and in vivo studies. AAPS PharmSciTech. 2007; 8:1.
20. New RRC. Preparation of liposomes and size determination. In: New RRC, editor. Liposomes-a practical approach. Oxford: Oxford University Press.,1990, p. 36-39.
21. Sandhya KV, Devi GS, Mathew ST. Liposomal Formulations of Serratiopeptidase: In Vitro Studies Using PAMPA and Caco-2 Models. Molecular Pharmaceutics., 2007, 5(1), 92–97.
22. Liang MT, Davies N, MToth I. Encapsulation of lipopeptides within liposomes: Effect of number of lipid chains, chain length and method of liposome preparation. International Journal of Pharmaceutics.,2005, 301, 247–254.
23. Solanki A, Parikh J, Parikh R. Preparation, Characterization, Optimization, and Stability Studies of Aceclofenac Proniosomes. Iranian Journal of Pharmaceutical Research., 2008, 7(4), 237-246.
24. Dubey V, Mishra D, Asthana A, Jain NK, Transdermal delivery of a pineal hormone:melatonin via elastic liposomes, Biomaterials. 2006, 27, 18, 3491–3496.