SHORT COMMUNICATIONS

Formulation and Evaluation of Ketoconazole Niosomes

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Ketoconazole niosomes were prepared by ether injection technique using surfactant (Tween 40 or 80), cholesterol and drug in five different ratios by weight, 1:1:1, 1.5:1:1, 2:1:1, 2.5:1:1 and 3:1:1. The niosomes were characterized for size, shape, entrapment efficiency and *in vitro* drug release (by exhaustive dialysis). The formulations were also tested for *in vitro* (cup-plate method) and *in vivo* antifungal activity (in rabbits) and compared with free ketoconazole. The results of the present study indicate that niosomes have the potential to reduce the therapeutic dose of ketoconazole by improving its performance.

Vesicle suspensions are promising dosage forms in dermal and transdermal drug delivery[1]. The use of vesicle suspension offers several advantages: the dosage form is a water based vesicle; the vesicle allows the incorporation of lipophilic and hydrophilic drugs and the characteristics of the dosage forms are variable in terms of vesicle composition, size, lamellarity and concentration. The use of non-ionic surfactant vesicles (NSV or niosomes) may have some advantages over phospholipid vesicles (liposomes) with respect to chemical stability, lower cost of chemicals and the large number of surfactant classes available to design these vesicular structures[2]. Non-ionic surfactants are chosen because they are less irritating to the skin than ionic surfactants[3]. Encapsulation of drug in niosomes can be predicted to prolong the drug action, enhance penetration into target tissue and perhaps reduce the toxicity[4-6].

Ketoconazole is a broad spectrum antifungal drug that is used topically in the treatment of candida or tinea infections of the skin. The present work deals with the preparation of ketoconazole niosomes by ether injection technique, its characterization, *in vitro* drug release and evaluation of antifungal activity.

Cholesterol was purchased from Qualigens Laboratories, Mumbai. Tween 40 and 80 were procured from S.D. Fine Chemicals, Mumbai. Dialysing membrane was purchased from Hi Media, Mumbai. All other chemicals and solvents were of analytical or pharmacopoeial grade. Ketoconazole was supplied as a gift sample by Guffic Pharmaceuticals, Mumbai.

Various niosomal formulations were prepared by slight modification of ether injection technique introduced by Deamer and Bangham.[7] For niosome preparation; Tween 40 or 80, cholesterol and ketoconazole were mixed in the ratio (by weight) of 1:1:1; 1.5:1:1, 2:1:1, 2.5:1:1 and 3:1:1, respectively. For each ratio, non-ionic surfactant (Tween 40 or 80) and cholesterol were weighed accurately and dissolved in 5 ml of chloroform. Ketoconazole (20 mg) was then dissolved in the lipid solution. The resulting solution was then taken in a syringe type of infusion pump and injected slowly into 30 ml of aqueous phase (phosphate buffer pH 7.4) held in a beaker maintained at 60-65° and agitated slowly. As the lipid solution was injected slowly into the aqueous phase, vaporization of chloroform resulted in the formation of niosomes. The ketoconazole-entrapped niosomes were separated from the unentrapped ketoconazole by dialysis of plain drug as discussed by Baillie *et al.*[8] The amount of ketoconazole dialysed was determined spectrophotometrically at 420 nm[9]. The size and shape characteristics of niosomes was studied under a high power microscope.

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The *in vitro* drug release from niosomal suspensions was studied by dialyzing the niosomal suspension in dialysis tubing. A glass tube of length 7 cm and diameter 2 cm was tied with a dialysis membrane at one end. The prepared niosomal suspension after the separation of free drug, was placed in it and the tube was suspended in 900 ml PBS (pH 7.4) maintained at 37±0.5° and stirred continuously at 100 rpm for a period of 24 h, after which the PBS was changed and diffusion of the same niosomal suspension into fresh PBS was carried out for 8 d. The diffused ketoconazole was estimated spectrophotometrically at 420 nm.

Each niosomal suspension after the removal of free drug was incorporated in FAPG Base (stearyl alcohol:20%, stearic acid:5% and propylene glycol:75%) to evaluate the *in vitro* antifungal activity by cup-plate method. The overnight grown culture of *Candida albicans* was inoculated into the sterilized potato dextrose agar media plates. After solidification, wells were cut into the media and fixed with 0.2 µg of the specimens to be tested using plain drug and marketed preparation of ketoconazole as control. The plates were incubated at room temperature and the width of the zone of inhibitions resulting after drug diffusion into media was measured.

On the basis of *in vitro* evaluation, three niosomal preparations, 2:1:1, 2.5:1:1 and 3:1:1 were selected for the evaluation of *in vivo* antifungal activity. Five healthy rabbits of either sex were selected. Lesions were developed by shaving an area of 2x2 cm on the back side of each rabbit to expose the skin. Four lesions were developed on each animal. Scarification was done with the help of scraper to an extent that bleeding should not occur and 0.1 ml spore suspension of *Candida albicans* was applied on the surface layers of skin. The development of infection was seen macroscopically on the third day as the area of reddening and erythema was accompanied by fine scale, crust and scab formation. The test formulations (0.2 mg) were then applied topically as a thin layer to the affected area, after every 24 h and 48 h interval respectively. The treatment was given for about eight days and the lesions were scored according to the following scale: 0-no reaction.,1-erythema, 2-scar/crust, 3-scab formation, 4-skin thickening and 5-redness and skin thickening. The study was approved by the Institute Animal Ethics Committee.

Ketoconazole niosomes prepared by ether injection technique employing varying ratios of surfactant (Tween 40 or Tween 80), cholesterol and drug were found to be spherical in shape and in the size range of 0.02-0.05 mm which is the size range of small unilamellar vesicles (SUVs). The size distribution of niosomes is shown in Table 1. The entrapment efficiency of drug was excellent (>95%) with all the niosomal preparations as shown in Table 2.

The *in vitro* release profile of ketoconazole from various niosomal preparations showed that using Tween-40 as surfactant, maximum drug was released from ratio 1:1:1 (65.25%) which had also shown maximum entrapment of ketoconazole. The release was more than 60% with all the niosomal preparations. Almost a similar release profile picture was observed when Tween-80 was used as a non-ionic surfactant, with ratio 1:1:1 showing 67% release and almost all the preparations showing more than 60% drug release. However, for both the surfactants, increase in the concentration of Tween 40 or Tween 80, decreases the drug release.

In vitro antifungal activity showed that after 18 h, Tween 40 niosomal preparation showed maximum zone of inhibition with ratio 3:1:1 than plain drug, marketed preparation and other niosomal preparations. Ratio 3:1:1 maintained its maximum activity after 48 h and 72 h as well. When Tween-80 was used as non-ionic surfactant, again ratio 3:1:1 showed better results over the other combinations, marketed preparation and plain drug. When the two non-ionic surfactants are compared for antifungal activity, Tween-40 niosomal

TABLE 1: SIZE DISTRIBUTION OF NIOSOMES.

Size range (µm)	Number of Niosomes									
	Tween-40					Tween-80				
	1:1:1	1.5:1:1	2:1:1	2.5:1:1	3:1:1	1:1:1	1.5:1:1	2:1:1	2.5:1:1	3:1:1
Below 0.05	8-14	12-16	5-9	17-21	15-19	12-14	15-18	7-10	15-17	13-17
0.05-0.07	83-85	80-82	90-92	81-82	75-77	70-73	74-76	82-84	79-80	72-75
Above 0.07	5-7	3-9	4-7	2-8	10-12	9-13	13-14	2-5	10-11	12-15

TABLE 2: ENTRAPMENT OF KETOCONAZOLE IN FORMULATED NIOSOMES.

Surfactant in niosomes	Ratio of surfactant, cholesterol and drug	Average ketoconazole dialysed (mg)	Percentage of ketoconazole in niosomes
Tween-40	1:1:1	0.5	98.0
	1.5:1:1	0.6	97.0
	2:1:1	0.4	98.0
	2.5:1:1	0.7	97.0
	3:1:1	0.7	96.0
Tween-80	1:1:1	0.7	97.0
	1.5:1:1	0.8	96.0
	2:1:1	0.6	97.0
	2.5:1:1	0.9	95.0
	3:1:1	0.8	96.0

Total amount of ketoconazole in niosomes used for dialysis studies is 20 mg.

preparations show better antifungal activity than Tween-80 niosomal preparations.

The *in vivo* studies revealed that, niosomal formulations show better healing than marketed preparation and plain drug preparations as shown in Table 3. Almost complete healing was observed around day 6 with most of the niosomal preparations. The results are correlated with the *in vitro* testing since prolonged effect of the drug is observed when encapsulated in niosomes. Tween 40 niosomes showed better healing rate. It appears from the present study that niosomes prepared by ether injection technique may be suitable carriers for local antifungal therapy by ketoconazole, increasing the therapeutic effectiveness by prolonged drug action.

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TABLE 3: COMPARATIVE *IN VIVO* ANTIFUNGAL ACTIVITY IN RABBITS.

Day	Time interval (h)	Marketed preparation	Plain drug preparation	SCORE					
				Tween 40			Tween 80		
				2:1:1	2.5:1:1	3:1:1	2:1:1	2.5:1:1	3:1:1
0	24	>4	>4	>4	>4	>4	>4	>4	>4
	48	>4	>4	>4	>4	>4	>4	>4	>4
2	24	4	4	3	3	4	4	4	3
	48	4	4	4	4	2	4	4	3
4	24	2	3	2	2	1	3	3	2
	48	4	3	2	1	1	3	2	1
6	24	1	2	1	0	0	2	2	1
	48	1	2	0	0	0	2	1	0
8	24	0	0	0	0	0	0	0	0
	48	0	1	0	0	0	0	0	0

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Spectrophotometric Estimation of Melatonin and Pyridoxine Hydrochloride in Combined Dosage Forms

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Simple colorimetric methods for the estimation of melatonin and pyridoxine hydrochloride in combined dosage form are described. Estimation of melatonin in presence ot pyridoxine hydrochloride is based on oxidative coupling reaction using 3-methylbenzothiazolin-2-one hydrazone (MBTH) and cerric ammonium sulphate. The reddish colored melatonin complex is measured at 530 nm against reagent blank it obeyed linearity over 1.2 to 15.6 μg/ml. Pyridoxine hydrochloride is estimated using ferric nitrate and 1,10-phenanthroline producing reddish brown colored chromogen, measured at wavelength of maximum absorption 510 nm against reagent blank. The chromogen obeyed linearity over 12.0 to 32.0 μg/ml for pyridoxine hydrochloride in presence of melatonin.

Chemically melatonin is N-[2-(5-methoxy-I-H-indol-3-yl)ethyl] acetamide[1]. It is recommended in psychiatric neurological and cardiovascular disorders[1]. It is not official in any pharmacopoeia and available in tablet form (Meloset tablets, Aristo Pharma). Literature survey reveals that the drug has been analyzed by voltametry[2], immunoassay[3], amperometry[4], electrophoresis[5], HPLC[6,7] and spectrophotometric methods[8]. Chemically, pyridoxine hydrochloride is 5-hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride[9]. It is used as a vitamin or a enzyme cofactor. It is available as pyridoxine hydrochloride powder[10-13], tablets[10,12] and injection[12-13]. Literature survey revealed that pyridoxine has been analyzed by non-aqueous[10,11,13], HPLC[12] and spectrophotometric methods.[10,12,13] Spectrophotometric methods were also reported for estimation of these drugs in combined dosage forms[14,15].

The present work describes simple colorimetric methods for estimation of melatonin and pyridoxine hydrochloride in combined dosage forms (Eternex, Dabur India Ltd.). A Systronic spectrophotometer 106 with 1 cm-matched cuvettes was used for spectrophotometric estimation. Cerric

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