RESEARCH PAPER

Accepted 23 July 1999
Received 31 March 1999
Indian J. Pharm.Sci., 1999, 61(6) 346-349

Development and Evaluation of Antifungal Activity of O/W type Creams Containing Solid Dispersion of Clotrimazole

B. MADHUSUDHAN*, D.RAMBHAU, V.R.GUDSOORKAR, J.S. SHETE AND S. S. APTE
G.Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad - 500 028

Clotrimazole (CLTZ) solid dispersions of various compositions were prepared by melting or melt-solvent methods using mannitol as carrier. Cream base of O/W type was prepared by emulsification method and the solid dispersion were incorporrated in cream base by trituration. The *in vitro* antifungal activity of CLTZ solid dispersion creams were estimated using the test organism *Candida albicans*. The interaction of sulphamethoxazole (SMZ) with CLTZ in the form of solid dispersion creams were also estimated for their antifungal activity. Impproved antifungal activity was found with CLTZ solid dispersions. However, there was no significant improvement in the activity in presence of SMZ.

Clotrimazole is an antifungal agent marketed as creams, ointments, lotions, solutions, powders and vaginal tablets. The major mechanism of action of CLTC against *Candida albicans* appears to be membrane action rather than their inhibition of ergosterol biosynthesis[1,2]. This would perhaps suggest that hydrophobicity of CLTZ is important. CLTZ is highly water insoluble and in an o/w cream its likely to remain partitioned in oil phase. The cream usually contains low drug concentrations of CLTZ such as 1% w/w. Because of this, efficacy of CLTZ cream is likely to be release rate limited. Thus an attempt to improve aqueous dispersibility will help to enhance the release from creams and it would be interesting to study its effect on efficacy of such formulations. Solid dispersion is one of the technique used to improve solubility, dissolution and bioavailability of several insoluble drugs[3,4]. To date some reports on formulations of these systems have appeared[4-13] mainly in tablets formulations. In this study, we attempt to evaluate the effect of solid dispersion of CLTZ formulated in a o/w type cream base on its antifungal activity.

EXPERIMENTAL

Solid dispersions containing clotrimazole-mannitol in the proportions of 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10 were prepared by melting and melt-solvent methods.

*For Correspondence:

Melting method[3]:

Solid dispersions were prepared by melting the physical mixture of clotrimazole and mannitol in a sandbath to about 160°. The molten mixture was immediately cooled and solidified in an ice bath with vigorous stirring. The solid mass thus obtained was scrapped, crushed, pulverised and passed through 60/80 mesh.

Melt-Solvent method[14]:

Clotrimazole (500 mg) was dissolved in chloroform (10 ml) and the solution was incorporated into the melt of mannitol by pouring slowly into hot melt with vigorous stirring. The melt was cooled immediately and the mass was kept under vaccum in a dessicator for 24 h. The solidified mass was scrapped, crushed, pulverised and passed through 60/80 mesh.

Characterisation of CLTZ solid dispersions:

Solid dispersions were characterised by studying their X-ray diffraction patterns and IR spectra. These were compared with those of physical mixture of drug and carrier. X-ray diffraction patterns were obtained by using Phillips PW 1010-1051 X-ray Diffractometer (Holland). All diffraction spectra were run at 2°/min in terms of a 2θ-

angle. IR spectra of CLTZ and solid dispersions were recorded in potassium bromide on Perkin - Elmer spectrophotometer model 283.

Preparation of Creams:

Creams were prepared by incorporating CLTZ plain or solid dispersion into a cream base of O/W type.

Preparation of Cream Base:

The base was prepared to contain Stearic acid 7 Parts, Cetyl alcohol 2 Parts, Mineral oil 20 Parts, Glycerin 10 Parts, Triethanol amine 2 Parts and Water q.s. 100 Parts by emulsification method. Stearic acid, cetyl alcohol and mineral oil were melted together at 60-70°. The aqueous phase containing glycerin, triethanol amine and water was heated to the same temperature. The aqueous phase was added to oily phase slowly with constant stirring and stirring was continued until cooled. The active constituent clotrimazole was incorporated as plain powder or as solid dispersion into the cream base by trituration. The concentration of clotrimazole was maintained at 1% of the cream. The creams were filled in collapsible tubes and stored for 24 h before evaluation.

Preparaton of clotrimazole - Sulphamethoxazole creams:

Solid dispersions of CLTZ-SMZ were prepared by melting method. A physical mixture of CLTZ-SMZ in 1:1 molar ratio was fused with mannitol in the proportion of 1:1, 1:2 and 1:4. The resultant solid mass after cooling was powdered and sieved through 60/80 mesh. The solid dispersions were incorporated in the cream base by trituration. The concentration of CLTZ was maintained at 1%.

Analysis of creams for clotrimazole:

Creams were estimated for clotrimazole content as per the standard method[15].

Determination of antifungal activity:

Creams were evaluated for antifungal activity *in vitro* using cylinder-plate method. The test organism *Candida albicans* was a clinical isolate obtained from local medical college hospital. Sabouraud's glucose agar medium was used for the study and for maintenance of stock culture. Cultures of organisms were maintained by periodically subculturing the organism on sabouraud agar slants. The slants after inoculation were incubated at 37° for 24 h and stored at about 5° in refrigerator. A loopful of 24 h agar slant culture was inoculated in 15ml of sterilized broth medium with the same composition. The tube was incubated at 37° for 24 h.

Freshly sterilized molten medium was cooled to 42-45°. The inoculum 1 ml/ 100 ml medium was added to the medium and mixed by rotating the flask slowly. About 30 ml of this seeded medium was poured into each of sterilized petridishes (10 cm in diameter) and allowed to solidify at room temperature on a horizontal surface.

Creams were filled in uniform sized plastic round cylinder (diameter 0.9 cm and length 0.8 cm) having both ends open, which were placed on the surface of agar medium in petriplates with the aid of forceps and pressed gently to ensure proper contact with the medium. Three cylinders were placed at equal distance in each petriplate. The plates were stored for 1 h at room temperature after which they were incubated at 37° for 24 h. The diameters of zones of inhibition formed were measured and the average calculated. Positive controls containing plain CLTZ or plain SMZ incorporated in a cream base and a negative control only cream base were tested along with the test samples.

RESULTS AND DISCUSSION

Solid dispersions of CLTZ in mannitol prepared by melting and melt solvent method were characterized by X-ray diffraction pattern and IR spectra. The characteristic peaks of CLTZ are observed at wave numbers 680, 700, 750, 760, 770, 1050, 1080, 1200, 1300, 1450 and 1500. The characteristic peaks of CLTZ and mannitol in physical mixture are also observed in solid dispersions. However there was a considerable decrease in the absorption intensities of solid dispersions prepared both with melting and melt-solvent methods (Fig.1). This indicates change from crystalline state to amorphus state. With increase in carrier concentration the reduction in the absorption intensities, reached maximum at a composition ratio of 1:4 (not shown in fig.) indicating highest amorphous state. The X-ray diffraction patterns depicted in fig.2 also indicate decrease in cristallinity. The characteristic drug and carrier peaks which are noticeable in physical mixture are greatly reduced in their intensities. These pattern of changes clearly prove a molecular level interaction between drug and carrier. The content of clotrimozole in creams is shown in table 1.

Table 1 shows the average zones of inhibition of various creams tested. All the creams containing solid dispersions have significantly improved antifungal activity

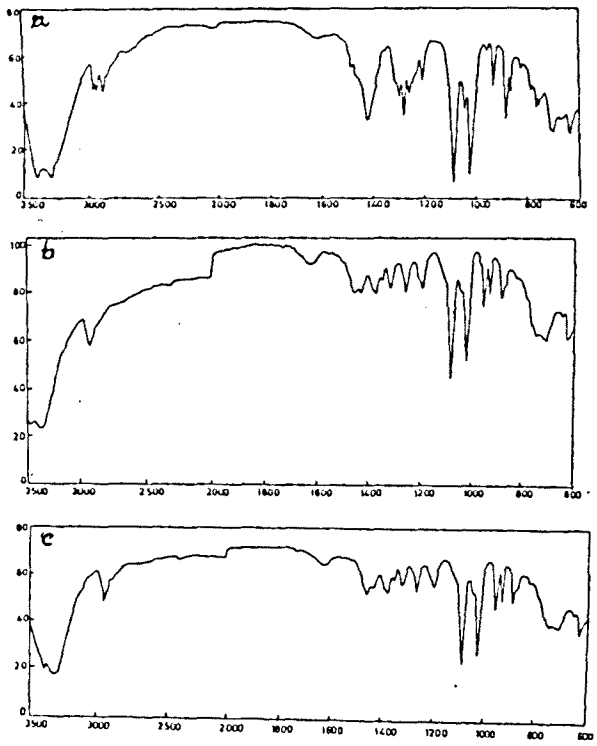

Fig. 1 : IR Spectra of CLTZ - Mannitol Solid dispersions in 1:6 ratio
(a) Physical mixture (b) Melting method (c) Melt-Solvent Method

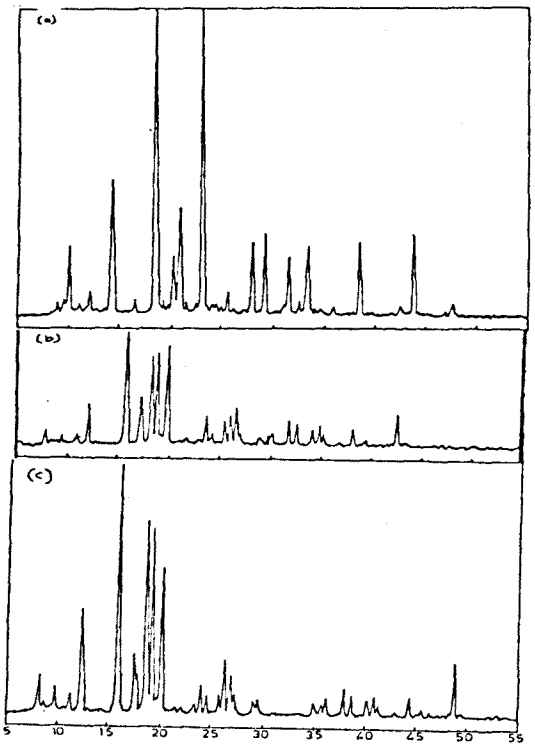

Fig. 2 : X-ray diffraction pattern of CLTZ-Mannitol Solid dispersions in 1:6 ratio
(a) Physical mixture (b) Melting Method (c) Melt-Solvent method

as compared to control. The highest activity was observed with 1:6 solid dispersions prepared by melting method. However, there was no significant difference in antifungal activity of creams prepared by melting and melt solvent method. The molecular interaction between drug and carrier results in association of several hydrophillic mannitol molecules with the drug molecule thereby attracting several water molecules to the near vicinity of insoluble molecular aggregates.

CLTZ, an antifungal drug used mainly against candidiasis is a hydrophobic drug. Surfactants like dioctyl sodium sulfosuccinate have been used to improve the dissolution of such drugs used for topical applications. Also cosolvents like glycerin or propylene glycol are incorporated in formulations to improve its activity. The molecular association of mannitol in the form of solid dispersion with CLTZ probably increases the aqueous dispersibility of microfined CLTZ particles. Due to this improved solubility, there is considerable increase in antifungal activity.

Sulphamethoxazole in concentration of 50 mg/ml in combination with CLTZ 1 mg/ml is reported to have exerted marked fungistatic effect on 4 different strains of *Candida albicans*[16]. The combined activity was interpreted as synergistic action. Based on this report we prepared creams containing solid dispersions of SMZ and CLTZ with mannitol as carrier. SMZ and CLTZ were incorporated in molar ratio of 1:1. The results of antifungal activity of these creams is shown in table 1. Though there was considerable improvement in antifungal activity of 1:1 solid dispersion, the activity was similar to that of control in solid dispersions with 1:2 and 1:4 ratio. The activity of 1:1 solid dispersion is comparable to that of 1:1 solid dispersion of CLTZ. Thus the activity appears to be solely due to CLTZ. Sulphamethoxazole may not have any influence on antifungal activity of CLTZ in the proportions used. In conclusion, the solid dispersion technique appears to enhance the activity of hydrophobic drugs such as CLTZ.

TABLE -1

CLOTRIMAZOLE CONTENT AND ANTIFUNGAL ACTIVITY OF CREAMS CONTAINING CLOTRIMAZOLE OR CLOTRIMAZOLE-SULPHAMETHOXAZOLE SOLID DISPERSION

	Drug-Carrier ratio	Amount of CLTZ present in 2 gms of creams (mgs)	Mean width of Zone of inhibition in cms.	±SEM
Cream base (As a negative control)			–	–
Plain CLTZ (As a positive Control)		21.00	1.95	0.15
Melting method	1:1	19.85	2.525	0.30
	1:2	19.50	2.22	0.07
	1:4	19.72	2.40	0.26
	1:6	19.00	3.10	0.57
	1:8	19.62	2.57	0.27
	1:10	19.02	2.47	0.22
Melt solvent method				
	1:1	20.04	2.37	0.16
	1:2	19.65	2.25	0.17
	1:4	19.00	2.25	0.06
	1:6	18.97	2.12	0.07
	1:8	19.00	2.35	0.20
	1:10	19.25	2.45	0.23
Melt method (CLTZ-SMZ based on their mole fraction)	1:1	20.06	2.62	0.44
	1:2	19.37	2.05	0.05
	1:4	19.09	2.00	0.00
Plain SMZ	-	-	-	

±SEM Standard Error Mean

REFERENCES

1. Hiratani, T. and Yamaguchi, H., **Chemotherapy**, 1984, 32, 829.
2. Hiratani, T., Uchida, K. and Yamaguchi, H., **Chemotherapy**, 1984, 32, 568.
3. Sekiguchi, K. and Obi, N., **Chem. Pharm. Bull.**, 1961, 9, 866.
4. Ford, J.L., **Pharm. Acta Helv.** 1986, 61, 69.
5. Youssef, M.K., El-Sayed, E.D. and Fouda, M.A., **Proceedings of 7th Pharmaceutical Technology Conference Liverpool, U.K.** April. 1988, 3, 51.
6. Szente, L., Apostol, I., Gerloczy, A. and Szejtli, J., **Pharmazie**, 1985, 40, 406.
7. Iwaoku, R., Arimori, K., Nakano, M. And Uekama, K., **Chem. Pharm. Bull**, 1982, 30, 1416.
8. Uekama, K., **J. Pharm. Soc.**, Japan, 1980, 100, 903.
9. El-Banna, H.M., Eshra, A.G. and Hammouda, Y., **Pharmazie**, 1977, 32, 511.
10. Ford, J.L. and Rubinstein, M.H., **Pharm. Acta. Helv.**, 1980, 55,1.
11. Ford, J.L. and Rubinstein, M.H., **Int. J. Pharm.**, 1981, 8, 311.
12. Ford J.L., **Pharm. Acta Helv.**, 1983, 58, 101
13. Meshali, M., El-Sabbagh, H. and Ramadan, I., **Acta Pharm. Fenn.**, 1983, 2, 173
14. Chiou, W.L. and Reigelman, S., **J. Pharm. Sci.**, 1971, 60, 1281.
15. United States Pharmacopoeia XXI - National Formulary, 16th Ed, United States Pharmacopoeial Convention, Inc., Rockville, 1984, 236.
16. Boggs, W.H., **Curr. Ther.Res.Chim.Exp.**, 1976, 20, 623.