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COMMUNICATION

Enhancement of Bioavailability of Griseofulvin by Its Complexation with β-Cyclodextrin

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ABSTRACT

Griseofulvin is a poorly soluble antifungal antibiotic drug, the solubility of which can be enhanced by complexation with β-cyclodextrin. The inclusion complex was prepared by coprecipitation method in various molar ratios of 1:1, 2:1, 3:1, and 1:2 of the drug and β-cyclodextrin, respectively. The inclusion complex was characterized and evaluated by UV–VIS spectral studies and FTIR. The in vitro drug release studies indicated that the 1:2 molar ratio complex form of the drug significantly increased the dissolution rate when compared to the free form. The acute toxicity studies clearly indicated that the β-cyclodextrin complex was nontoxic and the safety range was close to other Griseofulvin formulations. The in vivo study of the β-cyclodextrin was carried out in both animals and human beings by administering in four different rabbits and volunteers, respectively. Pellets made with Griseofulvin–β-cyclodextrin complex also showed a significant increase in the dissolution of the drug, revealing that β-cyclodextrin plays an important role in the solubilization of Griseofulvin.

INTRODUCTION

Pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate, chemical stability, absorption, and bioavailability. Cyclodextrins have received increasing attention in the pharmaceutical field (1). Cyclodextrins are cyclic malto oligosaccharides in which the glucose units are linked by α-1,4 glucosidic bonds (2). Because of the particular arrangement of the glucose units, the molecule has a cone-like structure which makes the exterior of the cone hydrophobic in nature, leading to formation of inclusion complexes with various drugs into its cavity and resulting in improvement in solubility and drug release (3). In the present work the interaction of β-cyclodextrin (β-CD) (which

has larger cavity size [7.5 Å] and better solubility than others) with Griseofulvin was investigated, with the aim of improving the aqueous solubility of Griseofulvin by extending its therapeutic employment and reducing its slight local irritant effect.

MATERIALS

Griseofulvin micronized USP was generously donated by American Remedies Ltd., Madras, India; β-CD, acetonitrile HPLC grade, methanol analytical reagent grade, and sodium lauryl sulfate were also used.

METHODS

Preparation of Griseofulvin–β-CD Complex

Solid complexes were prepared by taking different ratios of Griseofulvin–β-CD calculated on the molecular weight basis, by the coprecipitation method (4,5). A weighed quantity of Griseofulvin (drug) was dissolved in ethanol at room temperature to which required moles of β-CD in distilled water was added. The mixture was stirred by a magnetic stirrer at room temperature for 1 hr and then evaporated to dryness on a water bath. The inclusion complex precipitated as a crystalline powder was pulverized, sieved (#100), and stored in a desiccator.

Estimation of Griseofulvin in β-CD Complex

The pure drug and its complexes were estimated by spectrophotometer (7) and HPLC technique for in vitro and in vivo studies, respectively.

Evaluation of Inclusion Complexes

IR Spectral Studies

The spectra were obtained using a Bruker IFS 66v FTIR spectrophotometer in potassium bromide disk.

In Vitro Release of Complexes and Pure Drug

In vitro dissolutions of the pure drug and β-CD–drug inclusion complexes in pellet form were carried out in USP XXII Type II dissolution test apparatus using 900 ml of 0.54% w/v sodium lauryl sulfate in distilled water as a dissolution medium at 37 ± 0.5°C temperature, at a stirring rate of 100 rpm. Five-milliliter aliquots were collected at the time periods of 10, 20, 30, 45, 60, and 90 min. The aliquots were diluted to 50 ml with methanol–water mixture (4:1) and analyzed for the drug content spectrophotometrically at 291 nm.

Toxicity Studies of β-CD Complex in Animals

To assess the safety of 1:2 β-CD inclusion complex, the acute toxicity studies of the complex were carried out in Swiss Albino mice (8). Acute toxicity studies of β-CD inclusion complex were conducted in 30 Swiss Albino mice of either sex divided into three groups of 10 animals each. The complex was suspended in 0.05% of sodium carboxymethylcellulose and the solutions equivalent to 75, 150, and 300 mg/kg body weight at 5, 10, and 20 times the dose levels, respectively, were administered orally to respective groups of animals. After 72 hr, all animals were sacrificed and the postmortem naked eye macroscopic study was carried out for all of the vital organs (brain, heart, liver, kidney, lungs, stomach, spleen, intestine, and testis/ovaries).

In Vivo Studies

Since Griseofulvin–β-CD inclusion complex (1:2) was found to be nontoxic in Swiss Albino mice, the same complex was used for in vivo release studies by HPLC method (9); the mobile phase was prepared by degassing a mixture of 450 ml acetonitrile with 550 ml of 0.1 M acetic acid. Chromatographic analysis was carried out at a flow rate of 1.0 ml/min. The column temperature was ambient. The sample injection size was 10 μl and the column eluant was monitored by a UV detector at 290 nm.

In Animals

Four rabbits of either sex weighing between 2 and 2.5 kg were fasted overnight and dosed in the morning with 15 mg/kg drug in 0.05% sodium carboxymethylcellulose solution. Animal 1 received drug and it served as standard. Animals 2, 3, and 4 received Griseofulvin–β-CD inclusion complex. Blood samples (2 ml) were withdrawn from the marginal vein at time intervals at 0.5, 1, 1.5, 2, 3, 4, and 5 hr. Equal volumes of acetonitrile were treated with blood samples which were vortexed and centrifuged to obtain the clear supernatant solution. The solution was used to obtain the concentration of drug in the blood by HPLC method.

In Human Volunteers

To confirm the release study of prepared inclusion complex equivalent to 0.5 g of drug filled in capsules as a single dose was tested in healthy human male vol-

unteers and compared with the volunteer who received pure drug. Four healthy normal human male volunteers were fasted overnight. One volunteer received pure drug and served as a standard, the other three volunteers received 1:2 Griseofulvin–β-CD inclusion complex. Blood samples (2 ml) were withdrawn from the brachial vein by applying pressure on the cuff or the forearm at time intervals of 0.5, 1, 1.5, 2, 3, 4, and 6 hr. The samples were deproteinated using an equal volume of acetonitrile. The acetonitrile-treated samples were simply vortexted and centrifuged to obtain the clear supernatant. The content of drug was estimated by HPLC method.

RESULTS AND DISCUSSION

Evaluation of Inclusion Complexes

FTIR Spectral Studies

FTIR spectra of Griseofulvin–β-CD complex (A), Griseofulvin (B), and β-CD (C) are shown in Fig. 1. The spectrum of the Griseofulvin–β-CD complex reveals that there is a marked reduction in the intensity of the cyclic keto group at 1708 cm^{-1}, when compared to the spectrum of the pure drug. This may be due to hydrogen bonding between cyclic keto group (oxygen) of the Griseofulvin and secondary alcoholic hydrogen of β-CD.

In Vitro Studies

Release of Drug from Plain Griseofulvin Pellet

The percentage release of drug from plain pellets at the end of 30 min was found to be 14.4% in sodium lauryl sulfate medium and nearly 52.2% at the end of 5 hr.

Release of Griseofulvin from β-CD Inclusion Complex

As compared with pure drug pellet, the release rate of drug from the complex of 1:2 ratio was found to be gradual and almost follows the first-order kinetics rate release, and it showed a release of 97% within 45 min and 69% at the end of 5 hr (Fig. 2).

Toxicity Studies

The animals were kept under observation for 72 hr. At the end of 72 hr, all the animals were sacrificed and postmortem naked eye macroscopic studies were performed and compared with the vehicle-treated control group. No sign of toxicity was observed.

In Vivo Studies

In Animals

The in vivo study of β-CD was carried out by administering 15 mg/kg body weight of drug in four different rabbits. The time to reach peak serum concentration (6 μg/ml) of drug–β-CD complex was less than that of pure Griseofulvin. The pure Griseofulvin reached maximum peak at 4 hr (5.7 μg/ml) and β-CD complex reached maximum peak within 1.25 hr. This reveals that the absorption rate of drug was improved about four times when complexed with β-CD.

In Humans

In humans, the maximum drug concentration (7.0 μg/ml) from the β-CD complex was reached within 1 hr, whereas with the drug alone it was reached at 4 hr (6.63 μg/ml). This reveals that the absorption rate of drug was improved with the complex fourfold compared to the drug alone, probably because of the last dissolution rate of the complex. Thus, β-CD could be a useful additive to solid drug formulations because it may result in a more rapid and uniform release of the drug.

CONCLUSIONS

Griseofulvin is a poorly soluble drug and its absorption in the gastrointestinal tract is irregular. To enhance its solubility, complexation was made with β-CD in different ratios of (drug–β-CD) 1:1, 2:1, 3:1, and 1:2 by coprecipitation method. Drug content of complex was estimated by UV–VIS spectrophotometer at 291 nm and HPLC method. The complex was identified by FTIR and UV–VIS spectral studies. Dissolution studies were carried out in USP dissolution apparatus for in vitro release of all Griseofulvin and its complexes with β-CD, from which 1:2 complex showed improved solubility to other ratios. Toxicity studies were carried out to confirm the safety of 1:2 complex in Swiss Albino mice and it was proved that the β-CD complex was nontoxic and the safety range was close to other Griseofulvin formulations. In vivo studies were carried out in both animals and human volunteers to predict the availability of the drug in blood serum and it was confirmed that the drug–β-CD complex release was fourfold higher than that of pure Griseofulvin. This enhancement in the drug absorption could be attributed to the faster dissolution rate of the complex and its relative bioavailability was determined to be 73–84%. The results of this study indicated that Griseofulvin was com-

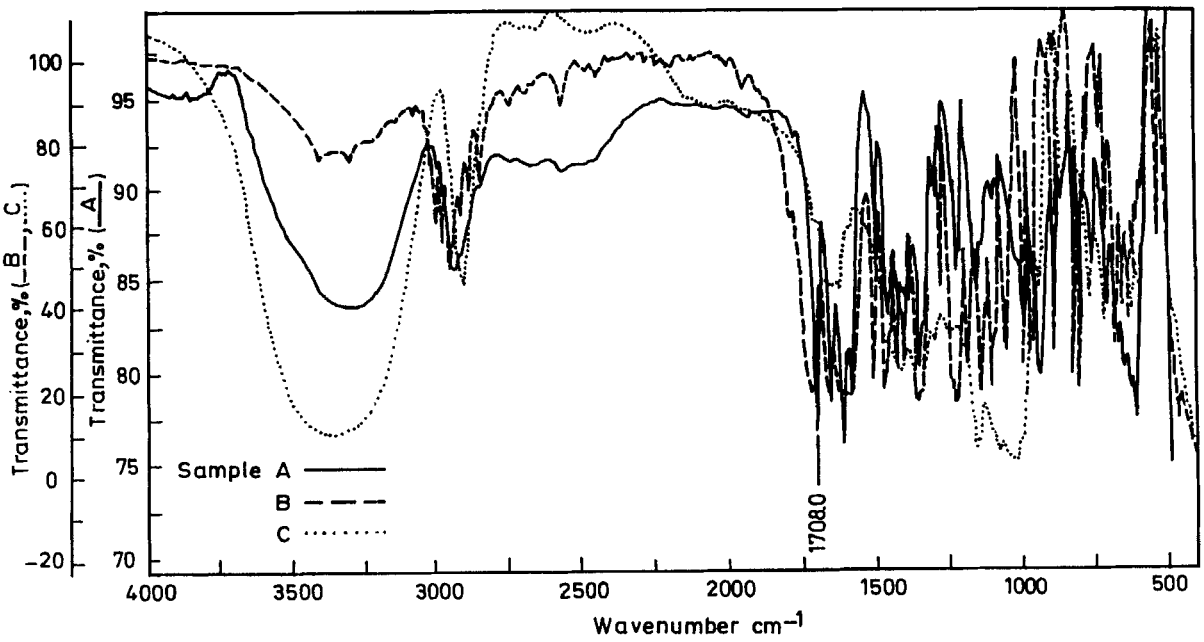

Figure 1. FTIR spectra of Griseofulvin–β-CD complex (A), Griseofulvin (B), and β-CD.

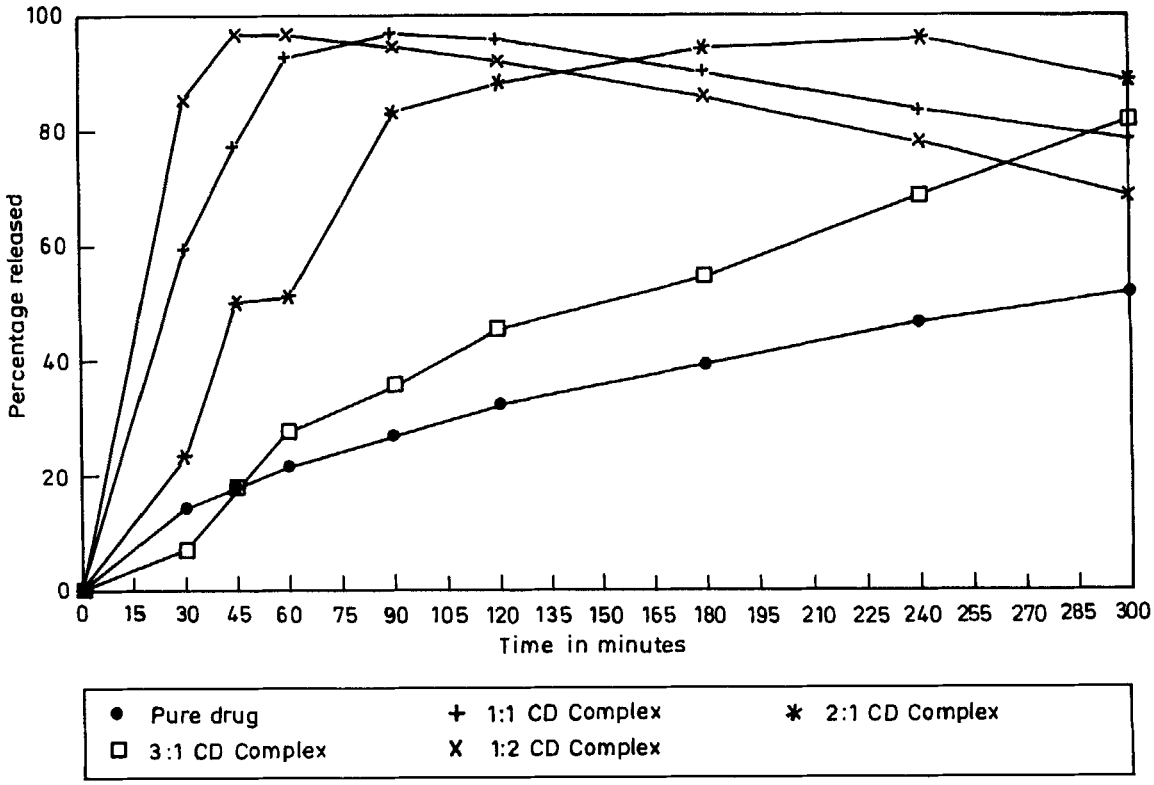

Figure 2. Release rate of Griseofulvin from inclusion complexes.

pletely available from its β-CD complex. Thus, β-CD could be a useful additive to solid Griseofulvin formulations because it may result in a more rapid and uniform release of the drug.

REFERENCES

1. J. Cohen and J. L. Lach, J. Pharm. Sci., 52, 132 (1965).
2. M. M. Maheswaran and S. Divakar, J. Scientific and Ind. Res., 53, 924 (1994).
3. J. Szejtll, Drug Invest., 2(suppl.), 11 (1990).
4. G. Puglisi, N. A. Santagati, and R. Pignatello, Drug Dev. Ind. Pharm., 16, 395 (1990).
5. N. M. Sanghavi, K. B. Choundhari, and R. S. Matharu, Drug Dev. Ind. Pharm., 19(16), 701 (1993).
6. B. S. Sastry, R. J. Venkateshwara, and C. S. P. Sastry, Indian Drugs, 28(1), 42 (1990).
7. *Pharmacopoeia of India*, Vol. 1, 237 (1985).
8. R. A. Turner, Screening Methods in Pharmacology, 60, 297 (1965).
9. W. J. Proveaux, J. P. Odonnel, and J. K. H. Ma, J. Chromatogr., 181, 77 (1980).