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Development and in vitro evaluation of griseofulvin gels using Franz diffusion cells

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Summary

The strong antidermatophytic action of griseofulvin, following oral administration, results from its concentration in the stratum corneum. However, this route of administration is often associated with various side effects. The purpose of this work was the preparation and in vitro evaluation of several gel formulations of griseofulvin, in which the drug was dissolved, for further clinical studies as an alternative topical dosage form. The in vitro release profiles of these formulations through artificial membranes and excised human skin, determined using Franz diffusion cells, showed that griseofulvin is released from the topical gel formulations employed and diffuses through skin. Additionally, stability studies conducted under room conditions for 16 months indicated that these formulations were adequately stable. Finally, preliminary clinical studies in humans showed that griseofulvin gels were effective and also well tolerated.

Introduction

Griseofulvin, which is known to be a very effective fungistatic antibiotic when administered orally, concentrates in the stratum corneum between 4 and 8 h after administration (Shah et al., 1974).

This oral route, however, is often associated with side effects such as headaches, gastrointestinal disturbances, blood dyscrasias, hepatotoxicity and gynecomastia (Martindale, 1982; Ritschel and Hussain, 1988).

Several investigators have attempted to evaluate the effectiveness of the topical route of administration. Zarowny et al. (1975) reviewed a number of positive and negative reports on the topical application of griseofulvin, and their results suggested that the drug may be effective following topical administration.

Skin concentrations resulting from a single topical application have been reported to be much higher than those obtained after prolonged oral administration (Epstein et al., 1975). Despite this observation and the fact that several investigators have shown the effectiveness of topically applied

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griseofulvin (Ritschel and Hussain, 1988) this route of administration remains within the area of experimental therapeutics. This may be due to inappropriate selection and in vitro evaluation of the vehicle which affects the availability of the drug for absorption and/or to a poorly designed clinical trial. Munro (1975) has pointed out that dimethylsulfoxide (DMSO), dimethylacetamide (DMA) and dimethylformamide (DMF) are very effective in delivering griseofulvin to its site of action, when the drug is applied as a solution in these solvents.

All the above observations suggest that further investigation should be carried out in order to evaluate the efficacy of topical griseofulvin formulations. The aim of the present study was the development of griseofulvin gels using two systems of solvents in which the drug is completely dissolved and stable. Furthermore, the release characteristics of these gels were evaluated in vitro using Franz diffusion cells with synthetic membranes and full-thickness human skin.

Materials and Methods

All the materials employed were used as received without further purification. Griseofulvin was kindly provided by Glaxo (Greece). DMSO was purchased from Merck (Germany), DMF from Carlo Erba (Italy) and polyethylene glycol 400 (PEG 400) from Hoechst (Germany). All were of analytical grade. Acetonitrile (HPLC grade-far UV) was obtained from Lab-Scan (Ireland).

Solubility studies

Excess griseofulvin was weighed and added to sealed conical flasks containing either solvent system I (10% DMSO, 80% PEG 400, H_2O q.s. 100%) or solvent system II (10% DMF, 80% PEG 400, H_2O q.s. 100%).

The flasks were agitated at 160 strokes/min for 7 days in a thermostated shaking water bath (Julabo, SW1, Germany) adjusted to 25 ± 0.5°C. The samples were then filtered through a 0.45 μm Millipore filter and the concentration of griseofulvin in each sample was determined by a modified high-pressure liquid chromatography (HPLC) method (Zia et al., 1979).

HPLC conditions

The HPLC system consisted of a high-pressure pump (Spectra Physics SP8800), a syringe loading sample injector (7125 Rheodyne) equipped with a Spherisorb ODS 2, S5 column (25 cm × 4 mm i.d.), a variable-wavelength detector (Spectra Physics, Spectra 100) and an integrator (Spectra Physics, Chromjet integrator). The mobile phase was prepared by mixing acetonitrile and water (6:4, v/v). The mixture was allowed to equilibrate at room temperature prior to filtration. The mobile phase (pH 3.4) was pumped through the column at a flow rate of 2 ml/min. The UV detector was set at 290 nm.

Preparation of standard solutions

The mother solution was prepared by dissolving a known amount of griseofulvin in acetonitrile. From this solution five standard stock solutions were prepared with appropriate dilutions in mobile phase. Calibration curves, constructed on the basis of peak height vs concentration, were found to be linear over the concentration range studied (correlation coefficients ranged from 0.992 to 0.999).

The method was adequately reproducible, with the percentage variation in the coefficients being within the range 4–7%.

Preparation of griseofulvin gels

Griseofulvin was dissolved at concentrations of 0.5 and 1.0% in solvent system I (formulations A1 and A2, respectively) and at the same concentrations in solvent system II (formulations B1 and B2, respectively). The solutions were gelled with 1.0% Carbomer 940 (Carbopol 940 B.F. Goodrich Chemical Co., U.S.A.), according to the technique referred to as thickening without neutralization (B.F. Goodrich Technical Bulletin GC-67, 1987).

Quantitative determination of griseofulvin in gels

Accurately weighed samples of gels were diluted to a certain volume with acetonitrile/water

(60:40) and stirred. Samples were filtered and injected into the HPLC system.

Stability studies

Stability studies were conducted under room conditions after the gels were filled in plastic transparent jars and aluminum tubes. The unchanged griseofulvin remaining was determined by HPLC at fixed time intervals, using the above-described method.

In vitro release studies

In vitro release studies were carried out using FDC 400 improved, open-cap flat-flange Franz diffusion cells with a 25 mm diameter orifice (Cell Drive Console, Crown Class Co., Inc., Somerville, NJ). In the donor compartment 3 g of the sample was applied and tapped down on a synthetic cellulose membrane (Visking Dialysis Tubing, 36/32 Serva, Germany) which was mounted between the donor and receptor compartments. The de-aerated receptor fluid was a mixture of PEG 400/DMSO/water (80:10:10), stirred by a bar magnet. The studies were carried out at 37 ± 0.5°C using three diffusion-cell assemblies. Samples of 50 μl were withdrawn via the side arm from each cell at appropriate time intervals (15, 30, 60, 120, 150, 180 min) and analyzed by HPLC.

In the case of formulation A2, full-thickness human cadaver skin was also employed for studying the rate of permeation of griseofulvin through skin. In the latter case, the sampling time intervals were 3.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h. All experiments were performed in triplicate.

Results and Discussion

From the solubility studies conducted according to the previously described method, it was found that the solubility of griseofulvin in both

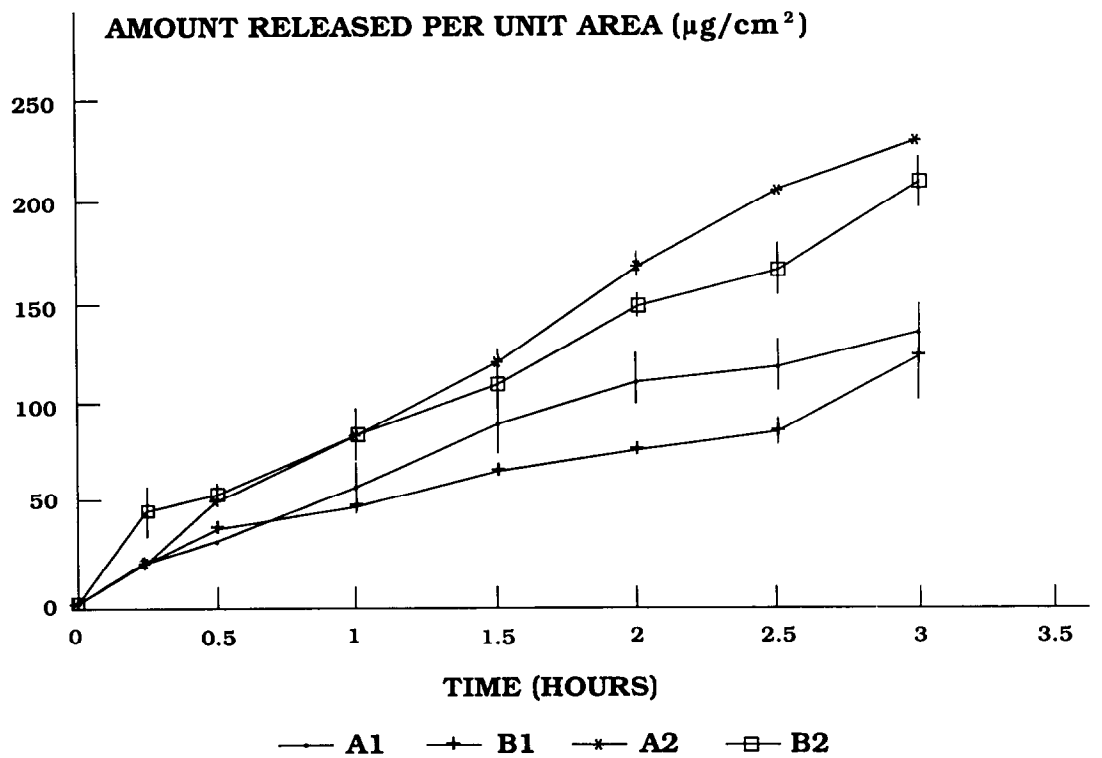

Fig. 1. Total amount of griseofulvin released (±SD) from formulations A1, A2 and B2 vs time.

solvent systems exceeded 1% (1.15% for solvent system I, 1.30% for solvent system II; significant difference at $p < 0.05$). Based on the above data the maximum concentration of griseofulvin incorporated into the gels studied was 1%.

The in vitro release profiles of griseofulvin from formulations A1, A2, B1 and B2, over a 3 h time period using the synthetic cellulose membrane are presented in Fig. 1. The amount of griseofulvin released per unit area ($\mu g/cm^2$) was plotted vs square root of time (Fig. 2). Using linear regression, the slope, which represents the release rate, was estimated.

The release rates (expressed in $\mu g/cm^2$ per $h^{1/2}$) for A1, A2, B1 and B2 were 97.8 ($r = 0.961$), 174.2 ($r = 0.992$), 72.96 ($r = 0.961$) and 132.34 ($r = 0.974$), respectively. The difference in the release rates of griseofulvin between gels A1 and B1 was significant at $p < 0.05$.

The same was observed for A2 and B2. The lower solubility of drug in solvent system I (10% DMSO) compared to II (10% DMF) facilitates its release (Idson, 1975).

A significant difference also exists between the release rates of the drug from formulations containing 0.5% griseofulvin (A1, B1) and 1% griseofulvin (A2, B2).

Doubling the drug concentration in the gel almost doubles its release rate. This finding is in agreement with the results of other investigators as summarized in a review article by Idson (1975).

The stability of all four formulations was tested under room conditions (25°C) in two different containers, with respect to percentage unchanged griseofulvin remaining after 16 months of storage. In most cases, there is less than a 3% decline in concentration of griseofulvin after 16 months of storage under room conditions. The type of container did not significantly affect the percentage of griseofulvin remaining. However, the formula-

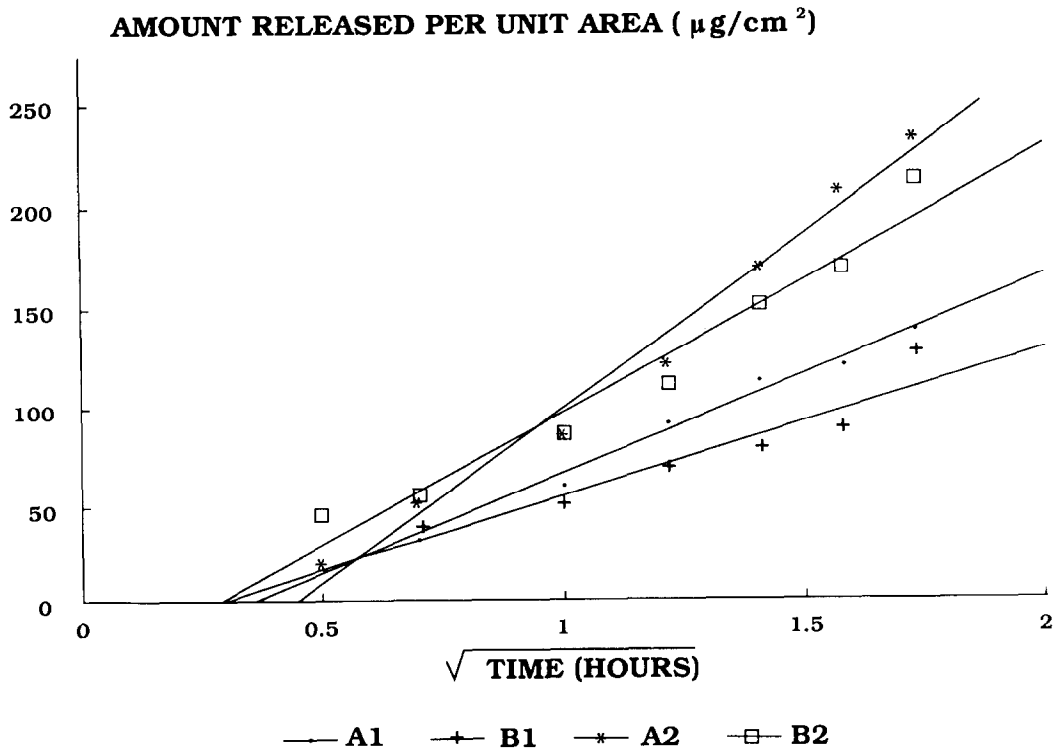

Fig. 2. Total amount of griseofulvin released from formulations A1, B1, A2 and B2 vs $\sqrt{\text{time}}$.

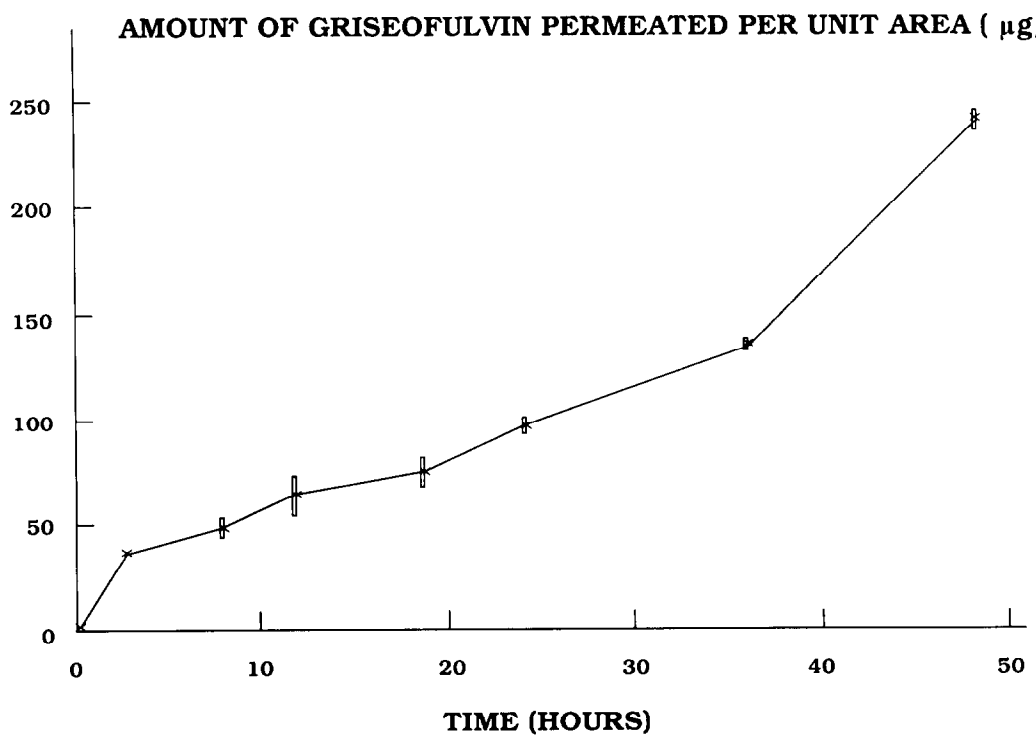

Fig. 3. Diffusion profile of griseofulvin (± SD), from formulation A2 across human skin.

tions B1 and B2 filled into transparent plastic jars exhibited a colour change from a colourless transparent to light-yellow transparent appearance.

Since formulation A2 was adequately stable, irrespective of the type of container, and shows the highest in vitro release rate, it was chosen as a vehicle for evaluating the diffusion rate of griseofulvin through full-thickness human cadaver skin. The diffusion profile of griseofulvin through skin is depicted in Fig. 3.

The results indicate that the drug diffuses through skin at an adequate rate compared with those determined in other similar reports (Epstein et al., 1975; Ritschel and Hussain, 1988).

The above formulation is currently under clinical investigation in human subjects, suffering from dermatophytoses of the skin in the 'A. Syngros University Hospital for Venereal and Skin Diseases'.

The results obtained to date demonstrate that a positive response to the treatment is found in more than 75% of the patients enrolled, while none presented any local or systemic side effects (Vlachou et al., 1991).

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