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Preparation of hydrogels of griseofulvin for dermal application

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Abstract

In an attempt to prepare topical formulations of griseofulvin that can deliver the drug locally in effective concentration, various hydrogel formulations were prepared using carbomer (940 NF) as base; essential oils, propylene glycol (PG), *N*-methyl-2-pyrrolidone (NMP) as penetration enhancers. The *in vitro* skin permeation studies through Laca mouse skin were performed using vertical type cells. PG in the hydrogel formulation was found to influence drug release rate by increasing its solubility and partitioning. Further combinations of PG with varying amounts of NMP in the hydrogel formulations exhibited a significantly greater increase in the flux on comparison with the control and formulation containing PG alone. The diffusion samples obtained by *in vitro* permeation studies through mouse skin when subjected to microbioassay using *Microsporum gypseum* as tester microorganism exhibited antifungal activity. This indicates that the drug permeated through the mouse skin possess sufficient antifungal activity *in vitro* against the tested microorganism. The prepared hydrogels did not show any skin sensitization and histological studies were carried out to check the safety of permeation enhancers used. Further these formulations were found to be stable at three different temperatures 4, 25 and 40 °C with respect to percent drug content, release characteristics, pH, transparency, feel and viscosity.

Keywords: Griseofulvin; Topical; Gels; Permeation enhancers; *N*-Methyl-2-pyrrolidone

1. Introduction

Topical drug delivery is an attractive route for local and systemic treatment. Griseofulvin is known to be a very effective oral fungistatic antibiotic. When administered orally it is concentrated in stratum corneum after 4–8 h of administration (Epstein et al., 1975). The need for a topical drug delivery system of griseofulvin arises due to its poor oral bioavailability because of its low water solubility and numerous side effects such as headaches, gastrointestinal disturbances, blood dyscrasias, hepatotoxicity and gynaecomastia (Ritschel and Hussain, 1988). Moreover, it has also been reported that skin concentration resulting from single topical application are much higher than those obtained after prolonged oral administration and persist there in measurable amounts for 4 or more days (Nimni et al., 1990). Researchers have reported that topically applied griseofulvin is a better prophylactic agent than either miconazole or clotrimazole (Wallace et al., 1977). Although, there are a number of recent reports on the topical formulations of griseofulvin, in which an attempt has been made to enhance the bioavailability of griseofulvin through the skin, by using variety of vehicles like dimethyl sulfoxide (DMSO), benzyl alcohol, acetone and isopropyl alcohol to dissolve the agent (Aly et al., 1994; Macasaet and Pert, 1994; Montes et al., 1994), with new carrier systems (Piemi et al., 1999; Pierri and Avgoustakis, 2005) and lately by use of penetration enhancers (Fujii et al., 2000) but none of the reported techniques has been yet established as clinically safe and efficacious to deliver the drug topically. Despite several investigations and numerous observations regarding the effectiveness of topically applied griseofulvin, this route of administration remains within the area of experimental therapeutics. This may be attributed to inappropriate selection and *in vitro* evaluation of the vehicle, which affect the availability of the drug absorption or a poorly designed clinical trial. This suggests further investigation of griseofulvin to reduce "body burden" of oral therapy.

Present work is an attempt to develop formulations of griseofulvin that are safe and can deliver the drug locally in an effective concentration. To achieve this we prepared topical hydrogel formulations using Carbopol 940 NF as a base. To enhance drug

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permeability absorption enhancers like eucalyptus oil, PG, NMP were added.

2. Materials and methods

2.1. Materials

Griseofulvin was obtained as gift sample from Shalaks Labs., India, *N*-methyl-2-pyrrolidone was purchased from E. Merck (India) Ltd., Carbopol 940 NF was obtained as gift sample from BF Goodrich, Germany, Bacto Yeast Nitrogen Base purchased from Difco Labs., MI, USA. All other reagents and chemicals were of analytical grade.

2.2. Preparation of carbopol hydrogels

Hydrogels of griseofulvin (0.2%, w/w) were prepared using Carbopol 940 NF (Pena, 1990; Wade and Weller, 1994). The Carbopol resin was soaked in water for 2 h and then dispersed by agitating at approximately 600 rpm with aid of mechanical stirrer to get a smooth dispersion. The stirring was stopped and liquid was allowed to stand so that any entrained air could escape. If any lumps of partially wetted Carbopol were present at this stage the dispersion was discarded and fresh batch was prepared. To this prepared dispersion aqueous solution of triethanolamine was added with slow speed agitation. At this stage, griseofulvin and the enhancers were incorporated into the prepared base by dissolving in ethanol. Ethanol in the preparation also served the purpose of preservative and no additional preservative was added. Any entrapped air in the gel was allowed to escape by allowing the gels to stand overnight. Table 1 shows the formulae of the topical preparations used in the study.

2.3. Evaluation of hydrogels

The prepared hydrogels were evaluated for percent drug content, drug uniformity, pH, skin sensitivity (Fisher, 1974), histological studies (Godkar and Godkar, 2003), viscosity and organoleptic characters such as feel tackiness, grittiness and transparency. Drug content was determined by taking 1 g of accurately weighed gel which was diluted to 100 ml with phosphate buffer pH 6.4 and analyzed spectrophotometrically at 296 nm. Viscosity studies were carried out using programmable Brookfield Rheometer fitted with spindle RV07 at 100 rpm and at temperature 25 °C.

Table 1
Composition of various hydrogel formulations of griseofulvin

Ingredients	Quantity (g), formulation code					
	Control	FI	FII	FIII	FIV	FV
Carbopol 940 NF	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	0.2	0.2	0.2	0.2	0.2	0.2
Ethanol	2.0	2.0	2.0	2.0	2.0	2.0
Eucalyptus oil	–	0.1	–	–	–	–
Propylene glycol	–	–	1.0	1.0	1.0	1.0
N-Methyl-2-pyrrolidone	–	–	–	1.0	2.0	4.0
Distilled water (q.s.)	10.0	10.0	10.0	10.0	10.0	10.0

2.4. In vitro permeation studies through mouse skin

2.4.1. Preparation of skin

Laca mice species was used for the study. The animals were sacrificed by overdose inhalation of chloroform. Hair on the dorsal side of the animals was removed with shaving razor in the direction of tail to head without damaging the skin. The shaven part of skin was separated from animals and hypodermis including blood vessels was removed using surgical blade no. 23. The excised skin was washed with distilled water and subsequently used.

2.4.2. Permeation studies

The permeation studies were carried out in quadruplicate, using mouse skin as a diffusion membrane. The diffusion cell used in the study was a modified Keshary and Chein diffusion assembly (Badkar et al., 2000; Proniuk et al., 2001). The receptor compartment had a volume of 35 ml. The surface area of the receptor compartment of assembly was 3.8 cm^2. Phosphate buffer pH 6.4 was used as receptor diffusion media. The cell contents were stirred by externally driven magnetic bar and were maintained at temperature of 37 ± 1 °C by circulating water through an external jacket of the cell. The different gel preparations were applied in an amount equivalent to 500 μg of drug on the membrane in donor compartment ensuring an intimate contact with the mouse skin. A 3 ml aliquot was periodically withdrawn at suitable time intervals from the sampling arm of receptor chamber. Fresh diffusion media was simultaneously replaced in the receptor chamber. The samples were analyzed spectrophotometrically at λ_{max} 296 nm.

2.4.3. Statistical analysis

All the data was statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's method.

2.5. Microbiological assay using disc diffusion method

A standard plot was prepared in the concentration range of 10–100 μg/10 μl of griseofulvin in DMSO. Sterile assay discs were spread in sterile petriplates and a 10 μl quantity of each of the prepared standard solutions was impregnated on to the discs. Then the discs were kept overnight at 37 °C for drying. For the preparation of nutrient agar plates 2 ml of YNBG media (with asparagine) was mixed with 18 ml of 2%, sterile molten agar and poured in the petriplates. The molten agar was then allowed to harden on a plane surface. The plates were incubated overnight at 37 °C and then inoculated with *Microsporum gypseum* spores (cell concentration used was 10^6 cells/ml). Swabbing of the petriplates with inoculum was done from edge to the center. Then the plates were dried for 30 min at 37 °C. The impregnated antifungal assay discs were placed on the inoculated nutrient agar plates with the help of sterile forceps. Finally, the nutrient agar plates were incubated at 25 °C for 72 h and zone of inhibition was observed. The samples obtained from *in vitro* skin permeation

studies of formulation IV and V were subjected microbiological test procedure in the same way as mentioned above.

2.6. Stability studies

The prepared hydrogels were subjected to stability studies in screw capped glass tubes at three different temperatures (4, 25 and 40 °C) and evaluated periodically (every week) for percent drug content, percent release characteristics, pH, transparency, feel and viscosity for 6 weeks.

3. Results and discussion

The hydrogels obtained were transparent, non-sensitizing, non-irritating and non-gritty. The drug content was found to be 99 ± 0.5%. Results of drug content uniformity test for all the gels indicated that the drug was properly and uniformly dispersed. The pH value of prepared gels was 7.2. The values for viscosity were found to be in the range of 22.6–28.5 Pa s and all the formulations were found to be easily spreadable and non-dripping in nature.

3.1. In vitro permeation studies through mouse skin

The permeation profiles from different formulations are shown in Fig. 1. The control formulation without any enhancer exhibited 84.51 ± 1.59 μg/cm^2 release in 12 h i.e., nearly 64% of the total amount. It may be due to the presence of ethanol in the formulation which was used to dissolve the drug before incorporation into the polymer base. The formulation containing 1% eucalyptus oil (FI) showed 95.57 ± 0.64 μg/cm^2 release in 12 h that was almost 8% more on comparison with control. But the rate of enhancement was only 1.24 (Table 2), which was not significantly different from control at $P < 0.05$. When PG was added as enhancer in the concentration of 10% (FII) 110.45 ± 1.69 μg/cm^2 was released in 12 h and enhancement ratio was significantly different at $P < 0.05$ when compared with control. Combinations of PG (10%) and NMP (1% and 5%) were also tried, but the amount permeated was similar to FII and the difference in value of enhancement ratio was statistically insignificant on comparison with FII (data not shown). It means that combination of PG and NMP at low concentrations is not contributing significantly towards penetration enhancement of the drug. Whereas, further higher concentrations of NMP (10%, 20% and 40%) in combination with PG (10%) gave four to six

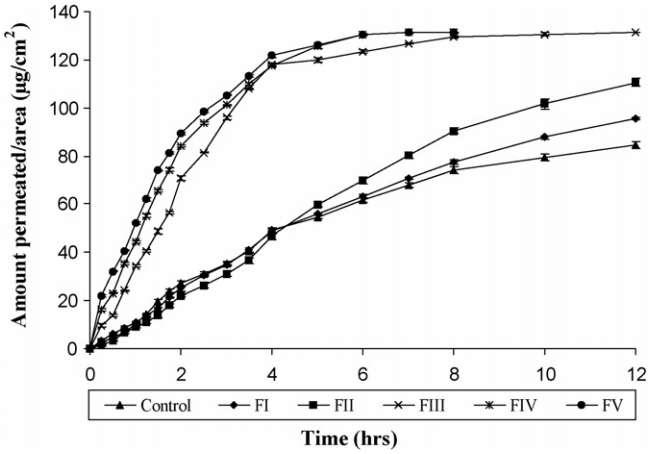

Fig. 1. Comparison of *in vitro* permeation profiles for control, FI, FII, FIII, FIV and FV through mouse skin.

Table 2
Comparison of flux and enhancement ratio of griseofulvin from different hydrogel formulations through mouse skin

Formulation code	Flux (μg/cm^2/h)	Enhancement ratio
Control	7.60 ± 0.13	–
FI	9.42 ± 0.02	1.24
FII	10.43 ± 0.16	1.37
FIII	31.38 ± 0.11	4.12
FIV	43.26 ± 0.06	5.69
FV	48.09 ± 0.46	6.32

times increase in flux, as compared to control. Such a high degree of enhancement in permeability of the drug can be attributed to a synergistic increase in solubility and partitioning of the drug by both the enhancers.

3.2. Microbiological assay

The antifungal activity of the drug was determined in terms of mean diameter of zone of inhibition. The diffusion samples obtained from skin permeation studies from selected formulations (IV and V) at time interval of 2, 4 and 6 h were subjected to microbioassay using disc diffusion method. These antifungal activity results obtained in terms of mean diameter of zone of inhibition upon conversion in terms of concentration were comparable to the concentration of griseofulvin determined spectrophotometrically (Table 3). Therefore, it can be said that the

Table 3
Comparison between concentration data obtained from microbiological assay and spectrophotometric analysis

Formulation code	Time (h)	Mean zone of inhibition ± S.D. (cm)	Amount of drug (μg) obtained from *in vitro* permeation studies	
			Microbioassay	Spectrophotometric analysis
IV	2	1.52 ± 0.083	8.97	9.12
	4	2.05 ± 0.050	12.35	12.78
	6	2.32 ± 0.112	13.73	14.26
V	2	1.58 ± 0.083	9.5	9.69
	4	2.12 ± 0.043	12.7	13.2
	6	2.42 ± 0.083	14.02	14.25

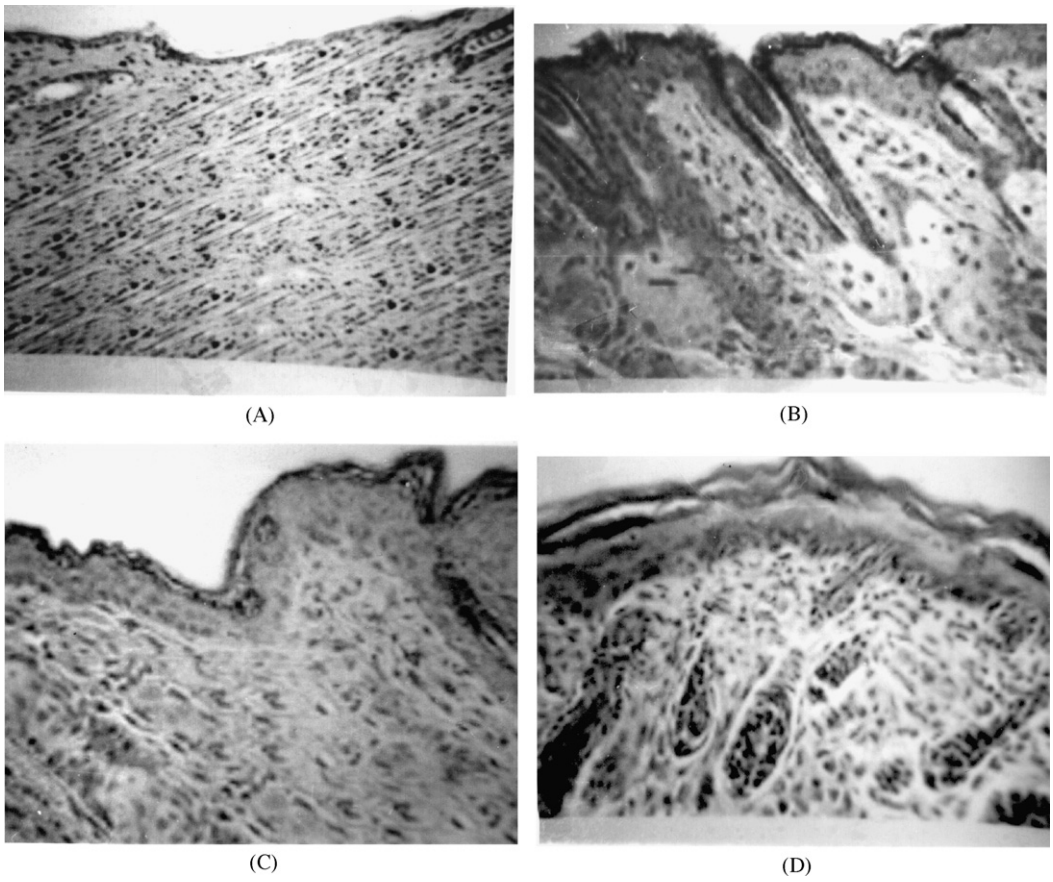

Fig. 2. Histological photographs of mouse skin after 4 h treatment with different formulations (original magnification 260×) (A) control, (B) formulation I (1% eucalyptus oil), (C) formulation IV (10% PG + 20% NMP), and (D) formulation V (10% PG + 40% NMP).

amount of drug permeated through mouse skin inhibited the growth of microorganism *in vitro*.

3.3. Histological studies

The aim of these studies was to establish the relative dermal tolerance of selected formulations FI, FIV and FV. The microscopic appearance of mouse skins without treatment and after treatment with FI, FIV and FV is shown in Fig. 2(A)–(D). Although, there was no change observed in the anatomical structure and no pathological changes were found but, slight increase in dermal hydration was observed in skin treated with FI and FIV and in case of FV an increase in stratum corneum hydration together with dermal hydration was also observed.

3.4. Stability studies

When the prepared griseofulvin hydrogel formulations were subjected to stability studies at three different temperatures (4, 25 and 40 °C), it was found that more than 99% of drug was present in all the formulations after 6 weeks at 4 °C, whereas at 25 °C there was about 2% and at 40 °C there was about 3% decline in the drug content in all the tested formulations after 6 weeks. Percent release profile of drug from the different formulations and pH remained unchanged after 6 weeks at all the temperatures. Other macroscopic characters like transparency, feel and viscosity were also observed and no significant change was found in these characters. This shows that the prepared formulations are stable at the studied temperatures.

4. Conclusion

The prepared griseofulvin topical hydrogels containing PG and NMP were observed to be safe, stable and very effective when tested on mice skin. A statistically significant amount of permeation was observed for formulations containing PG and NMP. This may be attributed to increased solubility and partitioning of drug from the formulations due to penetration enhancers. But these are only preliminary investigations on mouse skin. Further clinical studies are required to establish the efficacy and safety of these formulations on human skin.

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References

Aly, R., Bayles, C.J., Oakes, R.A., 1994. Topical griseofulvin in the treatment of dermatophytes. Clin. Exp. Dermatol. 19, 43–46.

Badkar, A., Talluri, K., Tenjaria, S., Jaynes, J., Banga, A.K., 2000. *In vitro* release testing of peptide gel. Pharm. Tech. 22, 44–51.

Epstein, W.L., Shah, V.P., Jones, H.E., 1975. Topically applied griseofulvin in prevention and treatment of *Trichophyton mentagrophytes*. Arch. Dermatol. 111, 1293–1297.

Fisher, A.A., 1974. Sensitivity testing. In: Balsam, Sagarin (Eds.), Cosmetics-Science and Technology, vol. 3. Wiley Interscience Publication, New York, pp. 283–310.

Fujii, M., Bouno, M., Fujita, S., Yoshida, M., Watanabe, Y., Matsumoto, M., 2000. Preparation of griseofulvin for topical application using *N*-methyl-2-pyrrolidone. Biol. Pharm. Bull. 23, 1341–1345.

Godkar, P.B., Godkar, D.P., 2003. Textbook of Medical Laboratory Technology, 2nd ed. Bhalani Publishing House, India, 1000–1004.

Macasaet, E.N., Pert, P., 1994. Topical (1%) solution of griseofulvin in the treatment of tinea corporis. Br. J. Dermatol. 124, 110–111.

Montes, L.F., Oakes, R.A., Pert, P., 1994. Topical griseofulvin in tinea versicolor: a double blind study. J. Am. Acad. Dermatol. 19, 43–46.

Nimni, M.E., Ertl, D., Oakes, R.A., 1990. Distribution of griseofulvin in the rat: comparison of the oral and topical route of administration. J. Pharm. Pharmacol. 42, 729–731.

Pena, L.E., 1990. Gel Dosage Forms: *Theory formulation and processing*, vol. 4. Marcel Dekker, Inc., New York, pp. 381–388.

Piemi, M.P.Y., Korner, D., Benita, S., Martey, J.P., 1999. Positively and negatively charged submicron emulsions for enhanced topical delivery of antifungal drugs. J. Control Rel. 58, 177–187.

Pierri, E., Avgoustakis, K., 2005. Poly (lactide)—poly (ethylene glycol) micelles as carrier for griseofulvin. J. Biomed. Mater. Res. 75A, 639–647.

Proniuk, S., Dixon, S.E., Blanchard, J., 2001. Investigation of the utility of an *in vitro* release test for optimizing semisolid dosage forms. Pharm. Dev. Tech. 6, 469–476.

Ritschel, W.A., Hussain, A.S., 1988. *In vitro* skin penetration of griseofulvin in rat and human skin from an ointment dosage form. Arzneimittel Forschung 38, 1622–1630.

Wade, A., Weller, P.J., 1994. Carbomer. In: Handbook of Pharmaceutical Excipients, 2nd ed. The Pharmaceutical Press, London, 71–73.

Wallace, S.M., Shah, V.P., Epstein, W.L., 1977. Topically applied antifungal agents: percutaneous penetration and prophylactic activity against *Trichophyton mentagrophytes*. Arch. Dermatol. 113, 1539–1542.