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Use of bioadhesive polymer to improve the bioavailability of griseofulvin

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Abstract

The absorption of griseofulvin in human with normal particle size is minimal and unpredictable. However, the absorption can be increased by the costly process of size reduction to microsize or ultramicrosize. This study is carried out to demonstrate that the addition of a bioadhesive polymer can greatly increase the bioavailability of griseofulvin with normal particle size form. Four formulations: A, 30 mg drug (mean particle size of 14 μm); B, 30 mg drug and 300 mg poly(acrylic acid) crosslinked with 2,5-dimethyl-1,5-hexadiene (PADH); C, 30 mg per 10 ml aqueous suspension; and D, 30 mg per 10 ml oil-in-water emulsion were employed in this experiment. The in vitro dissolution study using a Desaga flow system shows only a slight difference in their dissolution patterns except for formulation B in acidic medium. However, the in vivo absorption study indicates major significant differences between the above dosage forms. New Zealand white rabbits were orally administered with the above dosage forms and the blood samples were collected from the marginal vein at different time intervals for 24 h. The plasma concentrations were determined with a high performance liquid chromatography (HPLC). The result indicates that the addition of PADH to griseofulvin can increase the total absorption by 2.9-, 4-, and 2.9-folds when compared with drug powder, aqueous suspension and emulsion, respectively. The mechanism of improvement is probably due to the increase in gastro-intestinal transit time and the intimacy of the drug with the absorbing membrane brought about by the bioadhesive polymer. © 1997 Elsevier Science B.V.

Keywords: Griseofulvin absorption; Bioadhesive polymers; Bioadhesion; Increased; Bioavailability

1. Introduction

The absorption of sparingly soluble drugs is frequently associated by their limited solubility in the biological fluids of the gastrointestinal tract (GIT). This property will provide a chance for some drug particles to escape to the region of low absorption such as the large intestine before being absorbed. This could result in a significant fraction of the drug being wasted. Griseofulvin, an

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oral effective antifungal agent, is one of these drugs where this problem is very significant. This drug has a very low aqueous solubility and a slow dissolution rate and as a result it exhibits a slow, erratic and incomplete absorption from the GIT of lower animals and humans (Chiou and Riegelman, 1971; Kadir et al., 1986). For these reasons, research in this field has been directed towards enhancing the solubility and dissolution rate of this poorly absorbed drug in an effort to develop an improved and convenient griseofulvin dosage form. One of the known methods of increasing the rate of dissolution and absorption of this drug is by particle size reduction through micronization and ultramicronization. However, this process is considered expensive and often lead to aggregation and agglomeration of particles resulting in poor wettabilities. This problem has been minimized by solid dispersions of the drug with water-soluble carriers (Chiou and Riegelman, 1970), but the high surface energy of the particles produced tend to limit their physical stability. On the other hand, the enhancement of griseofulvin absorption in the presence of fat or triglycerides reported by Kabasakalian et al. (1970) led Carrigan and Bates (1973) to develop the oil-in-water emulsion. To date, this dosage form is considered as the best delivery system for this drug.

From the literature survey, it appears that the improved bioavailability of griseofulvin was mainly due to either promoting the drug dissolution or delaying the gastrointestinal emptying process which could increase the total amount of drug absorbed. Therefore, the use of bioadhesive polymers for the purpose of keeping the drug for a prolonged period of time in that region should be of general interest. It has been shown by several workers (Ch'ng et al., 1985; Hui and Robinson, 1985; Longer et al., 1985) that cross-linked polymers of acrylic acid can adhere to the mucus layer of the GIT resulting in the increase of transit time. The increase of gastric transit time will further improve the absorption of griseofulvin since it has greater solubility in the acidic medium. Polyacrylic acid crosslinked with 2,5-dimethyl-1,5-hexadiene (PADH) is one of these water swellable polymers which have been found to possess good bioadhesive properties (Ch'ng et al., 1985), hence by using this polymer as a drug carrier may offer the possibility of increasing the bioavailability of griseofulvin when compared with other dosage forms.

2. Materials and methods

2.1. Materials

Griseofulvin (mean particle size of 14 μm) and diazepam were purchased from Sigma. Acrylic acid, benzoyl peroxide, potassium dihydrogen phosphate, sodium dihydrogen phosphate-2-hydrate and sodium chloride were obtained from E. Merck (Darmstadt, Germany). Magnesium sulfate heptahydrate and phosphoric acid (BDH Chemicals, Poole, UK), acacia (Halewood Chemical, UK), palm oil PL 65 (Lam Soon, Selangor, Malaysia), 2,5-dimethyl-1,5-hexadiene (TCI, Kasei, Japan), anhydrous disodium hydrogen phosphate (R & M, Essex, UK), dichloromethane (J.T. Baker, NJ, USA), hydrochloric acid (Carlo Erba, Italy) and acetonitrile (HPLC grade, Labscan, Dublin, Ireland) were purchased from different sources. All the above chemicals were either reagent or analytical grades and were used as received.

New Zealand white rabbits weighing between 2.7 and 2.9 kg were used throughout the study. The animals were fed with soft food pellets with no restriction on the amount of food or water consumed.

2.2. Synthesis of the polymer

Polymer of acrylic acid cross-linked (0.3% w/w) with 2,5-dimethyl-1,5-hexadiene was synthesized according to the method of Ch'ng et al. (1985). At the end of the reaction, the polymer was repeatedly washed with distilled water to remove any unreacted substances and magnesium sulphate. The washed cross-linked polymer was dried in a hot air oven at 90°C for 24 h before being ground to the required size 400–630 μm.

2.3. Drug solubility studies

The solubility of griseofulvin in distilled water, simulated gastric and simulated intestinal fluids was determined at 37°C. An excess amount of griseofulvin (30 mg) was introduced into a vessel of a Sotax AT7 dissolution tester (Basel, Switzerland) containing 1000 ml of the each medium at 37 ± 0.2°C under continuous stirring with a paddle at 100 revs./min. After 24 h, 5 ml of the supernatant was withdrawn with a 5-ml volumetric pipette then filtered through a 0.45-μm membrane filter before its absorbance was determined by using a Hitachi spectrophotometer (Model D-2000, Tokyo, Japan) at 292 nm. By comparing with the standard plot constructed over the range of 5 to 25 μg/ml in each medium, the solubility of griseofulvin was determined.

2.4. Preparation of dosage forms

Four oral test dosage forms were prepared for subsequent in vitro and in vivo evaluation. The first dosage form was 30 mg of griseofulvin powder in capsule. The second preparation was a physical mixture of 30 mg of griseofulvin and 300 mg of PADH in capsule. The third preparation was an aqueous suspension containing 30 mg of griseofulvin mixed with 2 g of acacia and 10 ml of chloroform water. The fourth preparation was an oil-in-water emulsion containing 300 mg griseofulvin dissolved in 40 ml palm oil by gradual heating and 20 g acacia. A primary emulsion was first obtained then a sufficient quantity of chloroform water was added to make 100 ml. The emulsion was further refined with an Ultra-Turax T50 electric homogenizer (J & H, Germany). The mean particle size of the resultant emulsion was 4 μm, as determined by the Coulter Counter LS 100 (Coulter Electronics, UK). The emulsion was assayed for its drug content by dissolving a known amount of the preparation in absolute ethanol and subsequent measurement of its absorbance at 292 nm. Each absorbance value was converted to a concentration unit with the aid of standard curve constructed over the 0–10 μg/ml range.

2.5. In vitro drug release

The in vitro release of griseofulvin from each of the four dosage forms was determined by using a flow-through cell system (Desaga, Heidelberg, Germany). Sample equivalent to 30 mg of griseofulvin of each dosage form was placed into a flow cell without using a prefilter but with an attachment of a stainless mesh to prevent blockage. Five l of either simulated gastric or intestinal fluids, equilibrated at 37 ± 0.5°C and stirred with a paddle at 300 revs./min was used. The dissolution medium was circulated by a peristaltic pump at a rate of 100 ml/min from the reservoir through the flow cell then back to the reservoir. Five ml of sample was withdrawn at regular intervals from the reservoir for analysis. The total volume of medium in the system was maintained by replacing with an equal volume of medium which was pre-heated to the same temperature. The drug was assayed by measuring its absorbance at 292 nm after filtering through a 'Millipore' filter assembly (0.45 μm). The drug concentrations were determined by interpolation from a standard curve constructed from known concentrations of griseofulvin ranging from 0 to 10 μg/ml in simulated gastric or intestinal fluids. Each test was conducted in triplicate and the average percentage of drug released over time was calculated.

2.6. In-vivo absorption studies

The in-vivo studies were conducted in 10 healthy New Zealand white rabbits. Prior to the experiment, the animals were fasted in wire-bottom cages for 24 h with water allowed ad libitum but were muzzled to prevent coprophagy (Maeda et al., 1977). Each freshly prepared test formulation was administered to 5 rabbits in an oral dose equivalent to 30 mg griseofulvin with a washout period of one week between the treatments. The drug powder and the bioadhesive dosage form were administered in a capsule form (no. 3) by the aid of plastic rod, while a volume of 10 ml of aqueous suspension or emulsion form was administered by a syringe fitted with a metal tube. The animals were subsequently restrained in wooden boxes with free access to water but not food.

Approximately 2 ml of blood sample was collected from the marginal vein into a heparinized tube at 2, 4, 6, 8, 10, 12 and 24 h time intervals after dosing. Blood sample was also collected before dosing to serve as blank. The blood samples were immediately centrifuged and the aliquot of plasma obtained were kept in refrigeration until required for analysis.

2.7. Determination of griseofulvin in plasma

The drug contents of the plasma samples were assayed by using the HPLC method of Hackett and Dusci (1978). The chromatographic system consisted of a 305 model pump (Gilson, Villers Le Bel, France), a 115 model variable wavelength UV detector (Gilson, HWY., USA) set at 295 nm and a Rheodyne® 7161 injector valve (Cotati, California, USA), equipped with a D-2500 chromatogram integrator (Hitachi, Tokyo, Japan). The column used was a 4 × 250 mm LiChrosorb® RP-18 (5 μm) from E. Merck (Darmstadt, Germany) fitted with direct-connect refillable LiChrosphere® guard column (E. Merck, Darmstadt, Germany). The mobile phase was consisted of 45% v/v acetonitrile in 45 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid at a flow rate of 1.5 ml/min. A plasma volume of 1 ml was deproteinized with 20 ml of dichloromethane containing 5 μg diazepam as an internal standard. After shaking for 1 min, the sample was centrifuged at 2000 revs./min for 10 min. The aqueous layer was aspirated off and a 10-ml of the organic layer was taken to dryness under stream of nitrogen at 50°C. The residue was reconstituted with 100 μl of the mobile phase prior to the injection of 15 μl into the HPLC column. The drug concentration was calculated on the basis of peak height of the drug relative to that of the internal standard.

The assay was validated by spiking each drug-free plasma with a standard ethanolic griseofulvin solution in a concentration range of 0.2–1.5 μg/ml and 5 μg of diazepam. Each sample was examined by HPLC using the same conditions. Standard curves, recoveries and precision studies were performed with blank plasma samples spiked with the drug in a concentration of 0.2, 0.8 and 1.5 μg/ml.

2.8. Pharmacokinetic data analysis

The plasma data were analyzed for the total area under the plasma concentration/time curve (AUC) from 0–24 h by using the trapezoidal formula. The maximum plasma concentration (C_{max}) and the time to reach that concentration (T_{max}) were obtained directly from the curve.

2.9. Statistical analysis

The mean plasma griseofulvin level and the kinetic parameters of each experimental group were calculated. All results were presented as mean ± S.E. of 5 animals. Significant differences between groups were calculated by using Student's t-test for paired or unpaired samples.

3. Results and discussion

3.1. Solubility behavior

The solubility of griseofulvin in distilled water at 37°C was found to be 12.9 mg/ml which is in good agreement with the published value by Vojnovic et al. (1993). A similar results (11.3 μg/ml) was obtained in simulated intestinal fluid. On the other hand, the solubility of griseofulvin was significantly increased at the same temperature in simulated gastric fluid (23.4 μg/ml). The results demonstrated that griseofulvin dissolves better in the acidic medium of the stomach than in water or alkaline environment of the intestine. Obviously, the passage of the drug into the neutral or alkaline region of the GIT could result in a potential decrease in the dissolution and absorption rates. It follows that there is a clear advantage to be gained if drug particles were to be retained in the stomach for a prolonged period of time. This will give more time for the drug to dissolve in the stomach where its dissolution is optimal. We believe that bioadhesive polymers which attach to the mucin covering of the stomach, can offer this advantage and act as a carrier to retain the drug particles in this region.

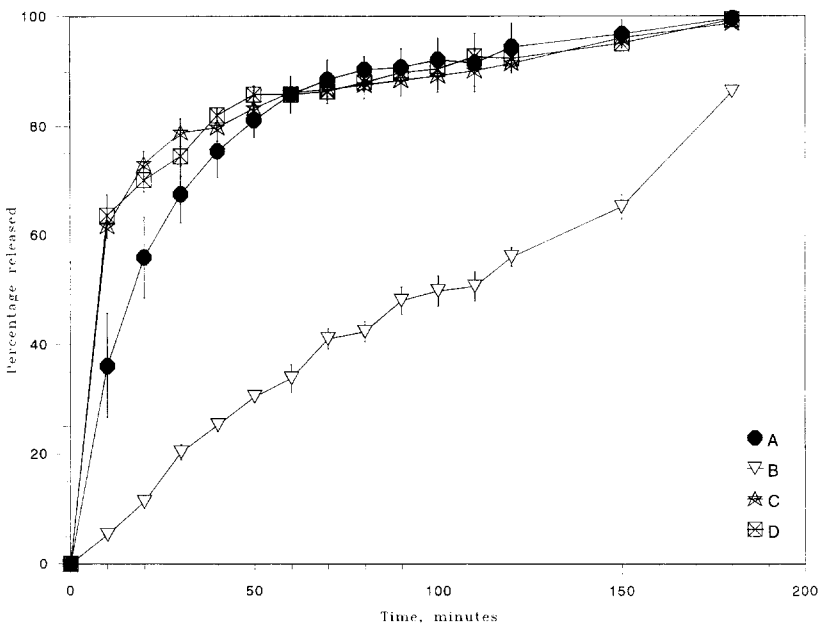

Fig. 1. In vitro release profiles of griseofulvin from various formulations in simulated gastric fluid. (A) Drug powder in a capsule; (B) drug with PADH in a capsule; (C) aqueous suspension; (D) oil-in-water emulsion. Each point represents the mean ± S.E. of triplicate experiments.

3.2. In vitro release studies

To conduct dissolution rate studies on a dosage form containing a barely water-soluble drug, it is important to select an appropriate experimental method. According to the solubility study of griseofulvin powder in distilled water, only ~ 43% of the total amount used could dissolved in 1 l at 37°C. It follows that at least 2300 ml is required to dissolve the whole dose (30 mg). This limited volume becomes critical with regard to the sink requirement which requires a sufficient large amount of solvent far in excess of the solubility limit. In this study, a volume of 5 l of the dissolution medium was chosen (~ 40% of the saturation) according to previous work by Chiou and Riegelman (1969) and Vojnovic et al. (1993) to run the test in a flow-through cell system in which different dosage forms were restrained in a small-volume cell and subjected to a stream of dissolution medium.

There were also other difficulties encountered during the initial trial of the dissolution study. The emulsion contents inclined to clog the prefilter in the sample holder, therefore, it was necessary to remove it before use. Instead, all the samples were filtered through a 0.45-μm pore membrane for subsequent assay. During the dissolution of the bioadhesive dosage form in the intestinal medium, it was observed that the bioadhesive polymer tends to absorb large amount of water and expands greatly in the limited space of the flow cell. Therefore, a stainless steel mesh was adapted to prevent the system blockage. For consistency of the experiment, the above two modifications were adopted for the all dosage forms tested in this study.

The release profiles from different dosage forms in simulated gastric and intestinal fluids are illustrated in Figs. 1 and 2, as the mean percentage of drug dissolved versus time. In the acidic medium, similar release patterns were observed for all formulations except that of a bioadhesive dosage form. The slow release of griseofulvin in the bioadhesive dosage form may due to the impediment of the flow of medium over the partly hydrated particles of the polymer which envelop the drug in the confined space of the diffusion

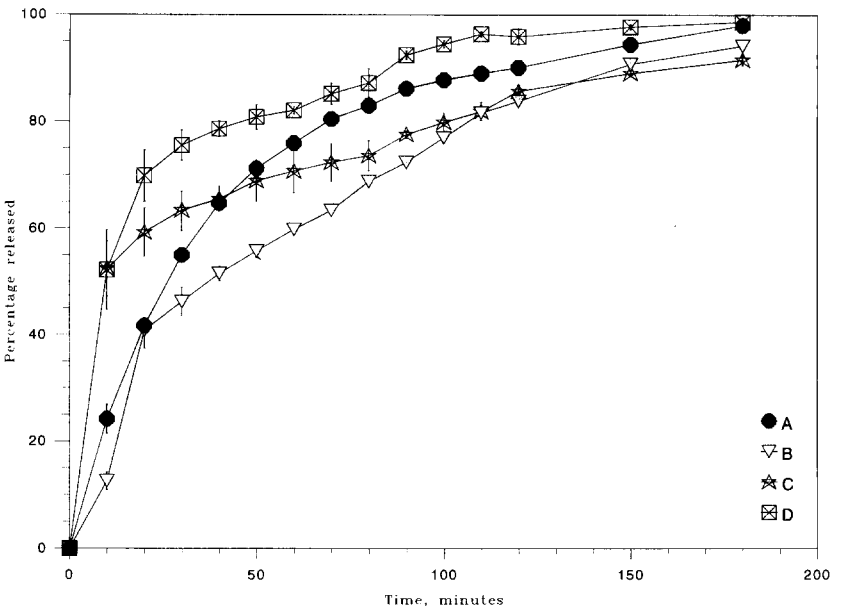

Fig. 2. In vitro release profiles of griseofulvin from various formulations in simulated intestinal fluid. (A) Drug powder in a capsule; (B) drug with PADH in a capsule; (C) aqueous suspension; (D) oil-in-water emulsion. Each point represents the mean ± S.E. of triplicate experiments.

cell. In contrast, the release rate is comparatively faster in the intestinal medium due to the extensive swelling of the polymer particles (Ch'ng et al., 1985) which become a loosely packed hydrogel and hence promote easier flow through of the medium. The variation of release patterns of other formulations in the alkaline intestinal fluid is more prominent with the emulsion providing the slight faster release of griseofulvin than the rest (Fig. 2). We can conclude that there is no significant difference in the in-vitro release of griseofulvin from the different dosage forms in gastric and intestinal fluids except for bioadhesive polymer in acidic medium.

3.3. Comparative bioavailability studies

The assay method used in this study was investigated for its sensitivity to detect the griseofulvin concentrations in the different plasma samples. The HPLC system gave base-line resolved peaks with retention times of 6.18 and 8.56 min for griseofulvin and diazepam, respectively. The assay was found to be specific and the blank plasma extract was clean and devoid interference at the retention time of griseofulvin. Standard curves constructed on the basis of the peak height ratios of griseofulvin to that of the internal standard from the rabbit plasma spiked with various ethanolic-drug concentrations were linear over the range of the plasma drug concentrations from 0.2 to 1.5 μg/ml. The correlation coefficient of 0.9997 and the constancy of response factors (peak height ratio divided by concentration) both indicate good linearity. In addition, the detector response determined with griseofulvin standards prepared in water with a concentration range of 0.2–10 μg/ml was found to be linear. The reproducibility of the method on a given day and day-to-day for 5 days was good. The within-day coefficient of variation was found to be 1.48% at 0.2 μg/ml, 3.78% at 0.8 μg/ml and 4.84% at 1.5 μg/ml. For between-day coefficient of variation values of the same concentrations were 2.01, 4.49 and 7.64%, respectively. The recovery study indicated that the extraction procedure employed was capable of quantitatively removing the drug from plasma with a mean value of 95.65% at 0.2 μg/ml,

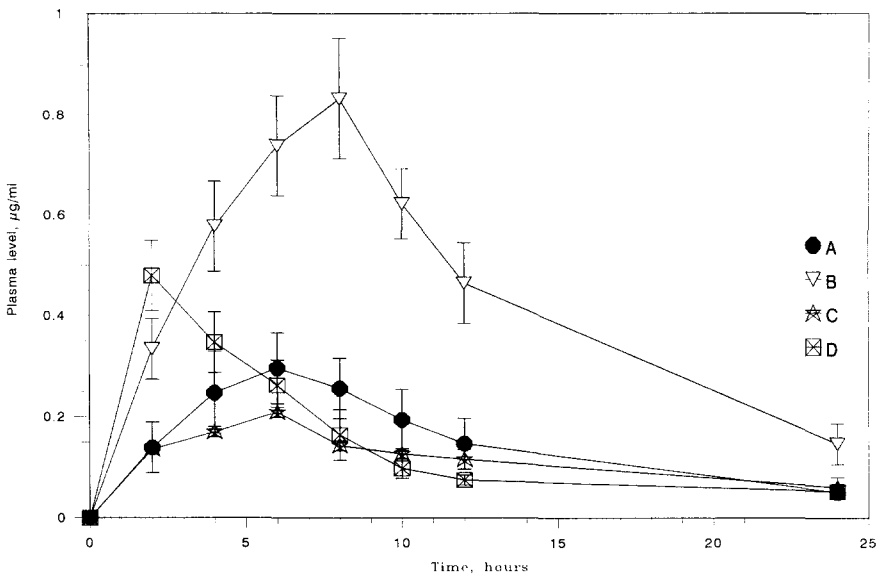

Fig. 3. Mean plasma griseofulvin level as a function of time after an oral administration of a 30-mg dose to rabbits. (A) Drug powder in a capsule; (B) drug with PADH in a capsule; (C) aqueous suspension; (D) oil-in-water emulsion. Each point represents the mean ± S.E. of five animals.

96.80% at 0.8 μg/ml and 97.57% at 1.5 μg/ml. The detection limit (4.5 times the noise level) for griseofulvin was 0.05 μg/ml. Twice the above concentration (0.1 μg/ml) which is seven times the noise level was found to be the lowest measurable concentration. This assay sensitivity was sufficient to follow the plasma level after an oral dose of 30 mg of griseofulvin in different test dosage forms for 24 h and allows the drug to be estimated down to a level of 0.1 μg/ml.

The mean griseofulvin plasma concentration versus time profiles in rabbits following an oral dose of 30 mg of griseofulvin in four test dosage forms are shown in Fig. 3. For all formulations, a minimum value was reached in 24 h, indicating that the experimental time period selected was adequate to assess the complete drug level and, thereby, the absorption characteristics of these formulations.

The plasma levels varied widely and declined at different rates as shown by the plots. There are also large variations in drug absorption between subjects on all formulations as indicated by the large error bars. Examination of this figure, together with the mean pharmacokinetic parameters in Table 1, revealed that a slight delay existed in the attainment of peak plasma drug level, T_{max}, following the oral administration of a bioadhesive dosage form as compared to drug powder, aqueous suspension and palm oil-in-water emulsion (8, 6, 6 and 2 h, respectively). However, the magnitude of the mean peak plasma level, C_{max}, of griseofulvin from a bioadhesive dosage form was approximately 2.8, 4.2 and 1.8 times higher than from drug powder, aqueous suspension and emulsion respectively. These differences were highly significant as determined by Student's t-test performed on the mean data (Table 2). Examination of the mean area under plasma-level time curve which reflect the amount of drug absorbed in 24 h, does not show any statistical significant differences between the drug powder, aqueous suspension and an emulsion. However, there is a highly significant 2.9-, 2.9- and 4-folds increase in the total amount of drug absorbed from the bioadhesive dosage form as compared to the drug powder, emulsion and aqueous suspension, respectively (Table 2). This enhancement of absorption can be attributed to the fact that the polymer can attach to the mucin covering of the stomach

Table 1
Pharmacokinetic parameters after oral administration of griseofulvin test dosage forms[a]

Dosage form[b]	C_{max} (μg/ml)	T_{max} (h)	AUC (μg h/ml)[b]
Drug powder in capsule	0.317 ± 0.07	6.0 ± 0.57	3.294 ± 0.92
Drug with PADH in capsule	0.881 ± 0.12	8.0 ± 0.57	9.454 ± 1.00
Aqueous suspension	0.209 ± 0.01	6.0	2.383 ± 0.18
Oil-in-water emulsion	0.479 ± 0.07	2.0	3.225 ± 0.49

[a]Data are presented as mean $\pm$ S.E. ($n = 5$)
[b]For 24 h after oral administration.

(Ch'ng et al., 1985) together with the drug particles for extended period of time. The detention of griseofulvin in this acidic condition can result in more griseofulvin being dissolved for absorption. The increase of intimacy of the dissolved drug with the absorbing membrane may also contribute to this effect.

In this study, the results indicated that the total amount of griseofulvin absorbed from the emulsion dosage form was not significantly different when compared with the aqueous suspension which is not in agreement with the results previously observed in the rat by Carrigan and Bates (1973) and in the human by Bates and Sequeira (1975) with the corn oil-in-water emulsion. They obtained a significant different in absorption between the two dosage forms and explained that the difference was due to the effect of emulsified oil and/or its digestion products (oleic acid and linoleic acid) on physiological process of GI motility and gall bladder evacuation. Although palm oil (PL 65) contains high concentration of oleic acid in triglyceride form (Tan, 1989), a similar increase in absorption of the drug was not observed. This may due to the difference in triglyceride contents of the oils and the variation in animal species.

Table 2
Statistical analysis of in vivo parameters obtained for various griseofulvin test dosage forms[a]

Formulation comparison[b]	C_{max}	T_{max}	AUC
A vs. B	S***	S*	S
A vs. C	NS	NS	NS
A vs. D	NS	S	NS
B vs. C	S	S**	S
B vs. D	S**	S	S
C vs. D	S***	S	NS

S, significantly different at $P < 0.001$ unless otherwise indicated; NS, not significantly different.
[a] Data were determined by Student's t-test.
[b] A, drug powder in a capsule; B, drug with PADH in a capsule; C, aqueous suspension; D, oil-in-water emulsion.
* Significantly different at $P < 0.05$.
** Significantly different at $P < 0.02$.
*** Significantly different at $P < 0.01$.

Although a dose level of 30 mg/rabbit (approximately equivalent to 10 mg/kg) which is the same as the recommended dose for antifungal therapy in dogs and man (Chiou and Riegelman, 1970) was used in the in vivo experiment, the blood levels of griseofulvin in the rabbits were found to be relatively low. This is probably due to the difference in the absorption capacity of the GIT and the metabolic rate of griseofulvin in this species (Maeda et al., 1979). As indicated in Table 1, the average peak levels, C_{max}, for all formulations used were not sufficient to produce the required therapeutic level which is estimated to be 1 μg/ml (Grin and Denic, 1973). Even the bioadhesive dosage form was only capable of producing a C_{max} of 0.881 μg/ml, hence in order to produce the required therapeutic level of 1 μg/ml griseofulvin, a dose higher than 10 mg/kg is required for rabbit.

4. Conclusion

On the basis of the results obtained, it can be concluded that the presence of a bioadhesive polymer increases the bioavailability of griseofulvin in rabbits, producing a satisfactory plasma concentration profile over 24 h. This significant improve-

ment may be accounted for by the delaying effect on the gastric emptying process caused by binding of the bioadhesive polymer to the gastric mucin/epithelial cell surface. This delay could have allowed only small amounts of undissolved drug to be emptied into the small intestine, as compared to the rapid emptying of the drug particles in the absence of a bioadhesive polymer. The prolonged detainment of drug particles with the bioadhesive polymer in the acidic medium of stomach would further promote the dissolution and absorption of griseofulvin.

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