Preparation of Griseofulvin for Topical Application Using N-Methyl-2-pyrrolidone

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We attempted to prepare a new griseofulvin formulation for topical application using N-methyl-2-pyrrolidone (NMP). Griseofulvin dissolves poorly in both water and oil, but dissolves in NMP to a concentration of about 100 mg/ml. A soybean oil–water emulsion with soybean lecithin and NMP as emulsifier and co-solvent, respectively, was prepared using a Microfluidizer, a high-pressure homogenizer. The size of the droplets in emulsion was about 200 nm, and the emulsion was stable for over 3 months. The skin permeation of griseofulvin through Yucatan micropig skin was studied in vitro using vertical type cells under donor phase open conditions. The permeation of griseofulvin from the NMP–water mixture (0–40%) into the skin tended to increase with increasing NMP concentration, although this finding was not statistically significant. Permeation from emulsion (oil phase, 20%; NMP 10–40%) was significantly higher than that from the water–NMP mixture. Permeation from the oil–NMP mixture was highest among the formulations investigated, and permeation from emulsion under donor phase closed conditions was significantly lower than that under open conditions. We believe that the evaporation of water from the emulsion after application to the skin was an important factor in skin permeation enhancement. When the emulsion containing 3% l-menthol was applied, a sufficient skin concentration (47 μg/cm² in dermis) was obtained.

Key words: griseofulvin; topical application; N-methyl-2-pyrrolidone; emulsion

Transdermal delivery is an attractive route for local and systemic treatment. However, permeation of drugs through the skin is insufficient for many therapies, especially in cases of sparingly soluble drugs. Griseofulvin, an antifungal agent, is one such drug, as it dissolves poorly in both water and oil, with a solubility in water of only 28 μg/ml. Although griseofulvin is usually used in the oral dosage form, its bioavailability changes easily according to particle size of the drug and diet. Topical application should prevent side effects, however the efficacy of griseofulvin after topical application has yet to be established. Recently, Nimmi et al. reported that application as a solution in benzyl alcohol, acetone and isopropanol provided effective penetration of griseofulvin and Aly et al. reported that topical use of this griseofulvin solution was effective against various types of dermatophytosis. These findings indicate that if a stable solution can be prepared, a sufficient concentration of griseofulvin will penetrate the skin for effective treatment of dermatophytosis.

In the present study, we attempted to prepare an oil in water griseofulvin emulsion for topical application. Soybean oil and soybean lecithin were selected as the oil phase and emulsifying agent, respectively. N-methyl-2-pyrrolidone (NMP), which has a similar solubility to dimethyl sulfoxide, was used as co-solvent, as it dissolves griseofulvin to a concentration of over 100 mg/ml and has a low incidence of skin irritation. The in vitro skin permeation of griseofulvin through Yucatan micropig skin from the emulsion was compared with other formulations.

MATERIALS AND METHODS

Griseofulvin was purchased from Sigma Chemicals. NMP (Pharmasolv™, Japanese Pharmaceutical Excipients grade) was provided by ISP Japan (Tokyo). Soybean lecithin (Basis LS-60, Nissin Oil, Tokyo, phosphatidylcholine content about 70%) and soybean oil (Nikka Yushi, acidic value<0.05, saponification value=191, Iodine value=132) were used without further purification. l-Menthol (JPXIII grade) was a kind gift from Kanebo Cosmetic Research Center (Kanagawa). All other chemicals were of reagent or HPLC grade.

Preparation of Sample The emulsion and suspension formulations are shown in Table 1.

Emulsion Griseofulvin and lecithin were dissolved in NMP, soybean oil was added and the mixture stirred until it became homogeneous. A third of water was then added to the mixture and pre-emulsification performed using a quick homomixer (Mizuho, Osaka) at 3000 rpm for 3 min. Residual water was added to the pre-emulsified emulsion with stirring, and the mixture then introduced into a Microfluidizer (Mizuho) and passed 10 times under a pressure of 10000 psi.

Suspension Griseofulvin was added to water or oil. In the NMP formulation, griseofulvin was dissolved in NMP, then water or oil added. The suspensions were kept overnight at 37°C.

Determination of the Size of Droplets in the Emulsion The mean diameter of droplets in the emulsion was determined by dynamic light scattering (ELS-800, Otsuka Electric, Osaka) after appropriate dilution with purified water. After the size distribution was confirmed as a single peak by histogram plot, the mean diameter was calculated using the cumulant method.

Skin Permeation Study A piece of Yucatan micropig skin (YMP skin set, Charles River Japan, Yokohama), after removal of the fat and subdermal tissue, was set on a modified Franz-type diffusion cell (area, 1.1 cm²; receptor, 16 ml of isotonic phosphate buffered solution). Two hundred microliters of the sample was poured into the donor phase and was changed every 12 h. The donor phase was open to the air to allow vaporization. The receptor phase was withdrawn at appropriate time intervals, and fresh solution added to maintain receptor volume. The duration of each experiment was 48 h.
Table 1. Formulations Used for Skin Permeation Studies

<table>
<thead>
<tr>
<th></th>
<th>Emulsion</th>
<th>Oil suspension</th>
<th>Water suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griseofulvin</td>
<td>0.1</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soybean lecithin</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>NMP</td>
<td>10</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Purified water</td>
<td>69</td>
<td>59</td>
<td>39</td>
</tr>
</tbody>
</table>

The drug concentration in the receptor phase was determined by HPLC.

**Concentration of Griseofulvin and 1-Menthol in the Skin** After the permeation studies, the skin was washed with purified water, wiped using Kim-wipe moistened by liquid paraffin. The skin was separated into epidermis and dermis using the heating method. Methanol was added to each sample, which was then homogenized (Hiscoxton NS-55 Nicholson, Chiba). After centrifugation, the concentration of griseofulvin and 1-menthol in the supernatant was analyzed by HPLC.

**Analysis of Griseofulvin** The concentration of griseofulvin was determined using HPLC (Shimadzu, Kyoto). The analytical system consisted of a pump (LC-6A), a UV detector (SPD 10A) operated at 294 nm and an integrator (C-R6A). Sample withdrawn from the receptor compartment was injected by an autoinjector (SIL 10A). The column (Wakosil-II 5C18HG, Wako Pure Chemical) was eluted at ambient temperature with 0.1 mol/l acetic acid: methanol (2:3) as the mobile phase, at a flow rate of 1 ml/min.

**Analysis of 1-Menthol** The concentration of 1-menthol was determined using HPLC (Shimadzu, Kyoto). The analytical system consisted of a pump (LC-10A), a RI detector (RID 10A) and an integrator (C-R6A). Sample was injected by an autoinjector (SIL 9B). The column (Wakosil-II 5C18HG, Wako Pure Chemical) was eluted at ambient temperature with 0.1% phosphoric acid: methanol (1:3) as the mobile phase, at a flow rate of 1 ml/min.

**RESULTS AND DISCUSSION**

**Preparation of Emulsion** The rate of dissolution of griseofulvin is known to be very low and a co-solvent is required to obtain sufficient permeation. Figure 1 shows the solubility of griseofulvin in the co-solvents commonly used for external preparations and mixtures with water. The solubilities in glycerin, propylene glycol and ethanol are about 0.1, 0.2 and 1.4 mg/ml, respectively, and thus they cannot dissolve sufficient griseofulvin if added to the emulsion. NMP dissolves griseofulvin to about 100 mg/ml, and thus appears to be good co-solvent for this drug.

Emulsions were prepared using 2.5—40% NMP as the co-solvent. When over 50% of NMP was used, emulsion could not be prepared. Figure 2 shows the size of droplets in the emulsion with or without 0.1% griseofulvin. The size of droplets without NMP was 280 nm, which decreased to around 200 nm with over 5% NMP. It is well known that amphiphilic solvents such as propanol reduce the interfacial tension and decrease the size of droplets. We also found that glycerin, propylene glycol and ethanol reduced the size of droplets. NMP has amphiphilic character, so it has the same function in the emulsion system. When griseofulvin was added, the size of droplets was also around 200 nm with over 10% NMP, and the size distribution was similar to that without griseofulvin immediately after preparation. However, griseofulvin precipitated from emulsions containing 2.5 and 5% NMP within 2 weeks. Emulsions containing 10—40% NMP were stable and showed no precipitate for at least 3 months. Thus, these formulations were used for the skin permeation studies.

**Permeation of Griseofulvin from Suspension in Water–NMP Mixture** Some investigators have reported an enhancement effect of NMP on the skin permeation of drugs. Thus, the effect of NMP on the permeation of griseofulvin was examined. The permeation profiles of
griseofulvin from suspension in various concentrations of NMP (Rp. W0—W40) are shown in Fig. 3. The permeation of griseofulvin from the water suspension was extremely low, at ca. 0.5 μg/cm² within 48 h. The amount that permeated the skin within 48 h tended to increase with increasing NMP concentration in formulation and that after application of Rp. W40 was significantly higher than that after Rp. W0. The concentration of griseofulvin in the epidermis tended to increase with increasing NMP concentration however this finding was not statistically significant. The concentration of griseofulvin in the dermis was independent of NMP concentration (Fig. 4).

Addition of NMP in water improved the solubility of griseofulvin, however NMP had no effect on the concentration of griseofulvin in the dermis. The increase in the amount that permeated the skin within 48 h appears to be the result of an increase in the diffusion coefficient of griseofulvin in the skin.

When griseofulvin was applied as a 100% NMP suspension, 6.8 μg/cm² of griseofulvin permeated the skin within 48 h, with the concentration in the dermis reaching 470 μg/cm³. However edema was observed, indicating irritation due to NMP itself or hypertonicity.

Permeation of Griseofulvin from Emulsion The permeation profiles from emulsions are shown in Fig. 5 and the skin concentration and amount permeated within 48 h are shown in Fig. 4 with the data of suspensions in water—NMP mixtures. The permeation of griseofulvin from emulsions was significantly higher than from suspensions, with the emulsion-derived griseofulvin concentration in the epidermis being about 3 times, and in the dermis about twice, that from suspensions with the same NMP concentrations. Thus, higher permeation from emulsions than suspensions was due to higher partition of griseofulvin in the skin.

The effect of NMP concentration in the emulsions was almost the same as that in the suspensions. The griseofulvin concentration in the epidermis and dermis tended to increase with increasing NMP concentration, however this finding was not statistically significant. Only the amount that permeated the skin within 48 h from Rp. E40 was significantly higher than that from other formulations.

The Effect of Application Method on the Permeation of Griseofulvin from Emulsion The above results suggested
Table 2. The Skin Concentration and Permeated Amount of Griseofulvin after 48 h Application of Oil Suspension

<table>
<thead>
<tr>
<th>Skin concentration (µg/cm²)</th>
<th>Amount permeated within 48 h (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epidermis</td>
</tr>
<tr>
<td>Rp. O0</td>
<td>2839±141</td>
</tr>
<tr>
<td>Rp. O0'</td>
<td>421±61</td>
</tr>
<tr>
<td>Rp. O33</td>
<td>7480±1440</td>
</tr>
<tr>
<td>Rp. O33'</td>
<td>33300±7100</td>
</tr>
</tbody>
</table>

that an important factor in skin permeation is not NMP concentration but dosage form. Emulsion seemed to improve the partition of griseofulvin in the skin. The donor phase was open to the atmosphere in these experiments, and thus the composition of the formulations would change with time. NMP concentration had little effect on the permeation of griseofulvin, and thus griseofulvin permeation from suspensions of water–NMP mixtures may not be affected by changing the formulation. To clarify the enhancement effect of emulsion, the donor phase was increased to 1 ml and closed with a glass ball to prevent a composition change.

The permeation profile within 36 h showed no difference between open and closed application, but from 36 to 48 h, permeation was lower when emulsion was applied under closed conditions. The skin concentration of griseofulvin was also significantly lower than that from the water suspension; 150 µg/cm² in epidermis and 5.2 µg/cm² in dermis. These results indicate that the enhancement effect of emulsion depends on the change in formulation after application.

Permeation of Griseofulvin from Suspension in Oil–NMP Mixture: Change in formulation appears to be important in enhancement of skin penetration of griseofulvin from emulsions. Among the ingredients in emulsion formulations, water is the most vaporizable. Thus, the permeation of griseofulvin from formulations expected to remain on the skin after evaporation of water was examined. The results are shown in Table 2. Permeation from Rp. O0 was almost the same as that from Rp. W0, and addition of lecithin (Rp. O0') had no effect on permeation. The addition of NMP (Rp. O33) significantly increased the skin concentration of griseofulvin and the amount permeated within 48 h. When the griseofulvin concentration was increased (Rp. O33'), the concentration in dermis was half that obtained after application of the 100% NMP suspension, although no skin irritation was observed.

Sasaki et al. reported that permeation of NMP itself from isopropyl myristate is higher than that from water. NMP is a relatively hydrophilic solvent, and thus is easily released from a hydrophobic solvent. Since soybean oil is more hydrophobic than isopropyl myristate, NMP might be released easily from soybean oil and partitioned to the skin, thereby dragging griseofulvin into the skin.

We believe that an emulsion applied to the skin changes its formulation upon evaporation of water, and thus NMP and griseofulvin easily partition in the skin, resulting in increased permeation of griseofulvin.

Combination with l-Menthol: High permeation and skin concentration was obtained when griseofulvin was applied as a suspension in an oil–NMP mixture. However, this mixture was not good feeling. To obtain higher permeation, we tried combination with an enhancer. Previously, we reported that a combination of NMP with l-menthol is effective for enhancement of indomethacin skin permeation. Thus, 3% l-menthol was added to Rp. E10. 3% l-menthol was added to Rp. W10 for comparison. The permeation profiles are shown in Fig. 6. The permeation of griseofulvin increased 7 times with l-menthol in both cases. The concentration in dermis increased twice, to 22.3 µg/cm² for Rp. W10 and 47.1 µg/cm² for Rp. E10. The permeation lag time seemed to be shortened by l-menthol. The concentrations of l-menthol in the skin after application as Rp. W10 and Rp. E10 were 560±260 and 440±190 µg/cm², respectively and there was no statistically significant difference. Thus, the enhancement factor (permeation (with l-menthol)) / (without l-menthol)) of l-menthol was the same in both formulations. It enhanced the permeation of griseofulvin in both formulations by increasing the partition of griseofulvin in the skin as twice and diffusion in the skin.

CONCLUSION

A stable emulsion containing griseofulvin was prepared using over 10% NMP as co-solvent. The permeation of griseofulvin through YMP skin from emulsion was higher than that from suspensions in water–NMP mixtures. High permeation from emulsion was due to a change in the composition of the formulation after application under donor phase open conditions. The concentrations of griseofulvin observed in the skin (14–46 µg/ml) are expected to inhibit fungal growth. In addition, we found that an emulsion containing l-menthol induces sufficient permeation and skin concentrations of griseofulvin, suitable for treatment of dermatophytosis.

REFERENCES