Enhanced Rectal Absorption of Amphotericin B Lyophilized with Glycyrrhizinate in Rabbits

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The influence of bases and additives in the formulation for rectal absorption of amphotericin B (AMB) lyophilized with dipotassium glycyrrhizinate (GLYK) was investigated using rabbits in relation to an in vitro release test. The release of AMB from the fatty base of Wittepol or a medium chain triglyceride (MCT) was markedly faster than that from the hydrophilic base of macrogol. The addition of polyoxyethylene (2) lauryl ether (POE(2)LE) into the fatty bases led to a marked increase in the release rate, whereas POE(9)LE or sodium lauryl sulfate resulted in significantly lower release rate. Animals received rectally each of seven AMB formulations of Wittepol H-15, macrogol, MCT with surfactants and aqueous solution. The absorption of the AMB lyophilized mixture with GLYK at a 1:9 molar ratio from a MCT base was significantly superior to that from macrogol. The addition of POE(2)LE into the MCT base resulted in a marked increase in bioavailability, showing the highest bioavailability of 4.9%. High serum levels of over 100 ng/ml of serum were maintained for 24 h following administration. The lowest bioavailability was 0.32% for the macrogol suppository. There was a good correlation between the release rate of AMB from the formulations and bioavailability. These results suggest that an AMB rectal formulation may provide a promising therapeutic alternative to infusion, taking into account the serum level of AMB exceeding the minimal inhibitory concentration of the infecting organism.

Key words amphotericin B; dipotassium glycyrrhizinate; rectal absorption; suppository; drug release; surfactant

Amphotericin B (AMB) remains the drug of choice for the treatment of deep-disseminated mycoses despite the severity of its adverse effects and its poor water solubility.1,2 In recent years substantial efforts have been paid to the development of new formulations3,4 and the synthesis5,6 of water soluble and less toxic AMB derivatives. However, most of these studies have been intended for the development of injectable dosage forms since the bioavailability of AMB and its derivatives in other dosage forms is believed to be considerably poor. To date, the bioavailability of AMB after oral administration has not been thoroughly evaluated.7 In addition, no data are available on the rectal absorption of AMB. While only one AMB intravenous form for systemic fungal infection is commercially available, a new dosage form of the drug other than an intravenous infusion is highly desirable to improve the convenience of use and to ease the adverse effects7 as much as possible.

Ching et al. studied the absorption of orally administered AMB lozenges in humans, suggesting that absorption across the buccal mucosa may be superior to the gastrointestinal absorption of AMB which is chemically unstable in the stomach.8 The rectal route can avoid the possible degradation in acid and therefore is considered to be preferable for improving poor oral bioavailability. Our previous studies9,10 revealed that AMB was absorbed rectally from a Wittepol H-15 base and the absorption was significantly enhanced by lyophilization with dipotassium glycyrrhizinate (GLYK). The present study was undertaken to explore the feasibility of further enhancement in the rectal absorption of the lyophilized AMB in relation to an in vitro release test. The pharmacokinetics of AMB after rectal administration were also discussed in comparison with those after intravenous administration in rabbits.

MATERIALS AND METHODS

Materials AMB (886 μg/mg as potency) and its commercially available AMB injection, Fungizone6, were obtained from Bristol-Myers Squibb Co., New Jersey, U.S.A. The amount of AMB is hereinafter expressed as its real potency. GLYK was purchased from Maruzen Pharmaceutical Co., Ltd., Hiroshima, Japan. Wittepol (type: H-15) and medium chain triglyceride (MCT), Miglyol-812, were supplied from Dynamit Nobel Co., Troisdorf, Germany. The MCT is a mixture of triglycerides which is composed of fatty acids with eight to twelve of carbon number. Macrogol 1000 and 4000 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used to prepare suppository bases. The following surfactants were added in the rectal formulations. Polyoxyethylene (2) lauryl ether (POE(2)LE), polyoxyethylene (9) lauryl ether (POE(9)LE) and sodium lauryl sulfate (SLS) were gifts from Nikko Chemical Co., Tokyo, Japan. Lecithin was purchased from Wako Pure Chemical Industries, Ltd. All other chemicals were of reagent grade.

Lyophilized Mixture of AMB and GLYK Lyophilization of the AMB and GLYK mixture at a molar ratio of 1:9 was performed according to the procedure described in the previous paper.10 All samples were stored in a refrigerator (5°C) until use. The lyophilized mixture and AMB crystalline powder were used after passing a 100 mesh screen.

Preparation of Rectal Formulations and Intravenous Injection Suppositories with Wittepol base were prepared by the fusion method, as described in the previous paper.10 Macrogol 1000 and 4000 were mixed at a weight ratio of 1:4 to form suppositories. A lyophilized mixture of AMB and GLYK or AMB powder alone was well dispersed in the suppository base after the mixture of PEG 1000 and 4000 had been melted in a beaker at 65°C. The molten mass was then
poured into a plastic mold (Kanae Co., Ltd., Osaka, Japan) and allowed to solidify at room temperature. Rectal formulations with MCT were prepared by dispersing a lyophilized mixture of AMB and GLYK with occasional stirring at 60 °C. The surfactant was added to the melted base at a concentration of 3% (w/w) to the weight of base. These formulations prepared were stored in a refrigerator (5 °C) and were used within 24 h after preparation. The aqueous solution for rectal administration was prepared by dissolving a lyophilized mixture of AMB and GLYK (1:9) in water to give an AMB concentration of about 15 mg/ml. The compositions of rectal formulations for the drug absorption test are presented in Table 1. In this drug absorption test, the 1:9 lyophilized mixture of AMB and GLYK was always used, since it previously showed the highest bioavailability of AMB. For intravenous administration, an aqueous solution was prepared by dissolving Fungizone® in water for injection. These solutions were prepared immediately before use. The amount of AMB incorporated was adjusted according to the body weight of the animals used.

**In Vitro Release of AMB from Rectal Formulations**

The release test was performed using a Franz-type diffusion cell according to the method reported by Hamamoto et al. Artificial membrane was utilized to prevent mixing or emulsification of the base with dissolution media. The membrane (Millipore, pore size; 5 μm) was placed at the top of the receptor chamber, and then sandwiched with the donor and receptor chambers. Phosphate buffer (0.05 M, pH 7.5, 60 ml) was placed in the receptor chamber. The receptor phase was stirred at 400 rpm with a magnetic stirrer bar. Then, 5 ml of the buffer was pipetted into the donor chamber. The top of the buffer was sealed with a glass cover slip to prevent evaporation of water. The assembly cell was placed in a 37 ± 1 °C environmental chamber. The release experiment was initiated by placing a unit of suppository or MCT formulation containing 30 mg of AMB per about 1.3 g of vehicle in the donor phase. One milliliter of sample was taken at specified time intervals, and the volume was replaced by fresh buffer equilibrated at the same temperature. The drug concentration in the receptor solution was determined according to the method of Nilsson-Ehle et al. In this in vitro release testing, the 1:9 lyophilized mixture of AMB and GLYK was always used.

**Animal Experiments**

White male rabbits, weighing from 2.6 to 3.2 kg, were fasted for a 24 h period before the experiments but they were allowed free access to water. The suppository was manually inserted into the rectum at 2.5 cm depth from the anus at the dose of AMB 10 mg potency/kg body weight. The aqueous solution or MCT formulation was given at the same dose using a plastic syringe. After insertion, the anus was fastened with a clip to prevent possible leakage. Intravenous injection was made into the marginal ear vein at a dose of 1 mg potency/kg body weight. Blood samples of 1—2 ml were collected from the ear vein at appropriate time intervals.

**Assay of AMB in Serum**

AMB in serum was assayed by high performance liquid chromatography as described in the previous paper.

**Pharmacokinetic Data Analysis**

Data on the serum concentration of AMB after intravenous administration were analyzed according to a two-compartment model, using the least-squares fit program, MULTI. The concentration of AMB as a function of time after administration can be described by the following equation.

\[ C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]

where \( C(t) \) is the drug concentration of time, \( t \) and \( \alpha, \beta \) are the biexponential equation constants. The half-life (\( t_{1/2} \)) of the terminal phase was calculated as \( t_{1/2} = 0.693/\beta \). The maximum serum concentration (\( C_{\text{max}} \)) and the time of its occurrence (\( t_{\text{max}} \)) after rectal administration were observed for each serum concentration—time curve. The areas under serum concentration—time curves from 0 to 24 h (\( AUC_{0-24h} \)) after intravenous or rectal administration were calculated by the trapezoidal rule. The absolute bioavailability of rectal administration (\( F \)) was calculated using the following equation:

\[ F = \frac{(AUC_{\text{recta}}/AUC_{\text{v}}) \times (D_{\text{recta}}/D_{\text{oral}})}{AUC_{\text{oral}}/AUC_{\text{v}}} \]

where \( AUC_{\text{recta}} \) and \( AUC_{\text{v}} \) are the rectal and intravenous areas under the curve, respectively, and \( D_{\text{recta}} \) and \( D_{\text{oral}} \) are the rectal and intravenous doses, respectively. In terms of \( C_{\text{max}}, AUC_{0-24h} \) and \( F \) values, statistical analysis was performed by using the one-way ANOVA, and the differences were assumed to be significant when \( p<0.05 \).

**RESULTS AND DISCUSSION**

**In Vitro Release of AMB from Rectal Formulations**

Figure 1A shows the release profiles of AMB from bases of rectal formulations in which AMB crystalline powder alone or the lyophilized mixture of AMB and GLYK is incorporated without surfactants. The release rates of the lyophilized AMB in MCT, Witepsol and macrogel bases were considerably enhanced in comparison with that of AMB crystalline powder. The release enhancing effect by lyophilization is likely due to the improvement of surface wettability of the drug particle and the solubility, as indicated previously. The release amount of the lyophilized AMB was significantly larger from the lipophilic bases like MCT and Witepsol than that from the hydrophilic macrogel base. The MCT and Witepsol bases released almost 10% and 4% of the total amount of the drug in 10 min, respectively, followed by 72.3% and 8.4% in 120 min. The extraordinarily faster drug release from the MCT base, which is given as a liquid form, seems to be due to a well-dispersed or partly dissolved con-

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**Table 1. Composition of AMB Formulations for Rectal Administration**

<table>
<thead>
<tr>
<th>Formation No.</th>
<th>AMB (mg)</th>
<th>Surfactant</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name</td>
<td>Amount (mg)</td>
<td>Name</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>—</td>
<td>MCT</td>
</tr>
<tr>
<td>2</td>
<td>POE(2)LE</td>
<td>37</td>
<td>MCT</td>
</tr>
<tr>
<td>3</td>
<td>Lecithin</td>
<td>37</td>
<td>MCT</td>
</tr>
<tr>
<td>4</td>
<td>POE(2)LE</td>
<td>37</td>
<td>WH15</td>
</tr>
<tr>
<td>5</td>
<td>SLS</td>
<td>37</td>
<td>WH15</td>
</tr>
<tr>
<td>6</td>
<td>SLS</td>
<td>—</td>
<td>Mac2</td>
</tr>
<tr>
<td>7</td>
<td>SLS</td>
<td>—</td>
<td>Ag.sote</td>
</tr>
</tbody>
</table>

(a) As potency, AMB lyophilized with GLYK (1:9, molar ratio) was used. b) Polyoxyethylene (2) lauryl ether. c) Sodium lauryl sulfate. d) Medium chain triglyceride. e) Witepsol H-15. f) Macrogol (1000/4000=1/4, w/w). g) Aqueous solution.
dition in the liquid phase. In contrast to the above, macrogol released only 0.6% in 120 min, despite the fact that the base and the lyophilized AMB well dissolved in water. While Witepsol and macrogol are given as solid form, the bases need to liquefy before releasing the drug. However, the time required for liquefaction of Witepsol or macrogol was short enough for drug dispersion or dissolution: Witepsol melted within 7 min and macrogol completely dissolved within 16 min. The slow release observed in the macrogol base is most probably due to the strong affinity of the drug to macrogol, which results in hindered migration of the AMB molecule in the dissolution media.\(^5\)

Figure 1B shows the release profiles of AMB from bases of rectal formulations in which the lyophilized mixture of AMB and GLYK was incorporated. POE(2)LE, POE(9)LE and SLS used in this study possess hydrophilic-lipophilic balance (HLB) values of 9.5, 14.5 and about 40, respectively. The addition of POE(2)LE to MCT and Witepsol bases enhanced the release levels to 96.4% and 46.0% from those of 72.3% and 8.4% in the base alone in 120 min, respectively. This is probably because the surfactant brought in a moderate hydrophilicity to the bases. On the other hand, POE(9)LE and SLS suppressed the release, suggesting that the longer polyoxyethylene moiety of the nonionic surfactant is likely to play a reducing role in a way similar to macrogol and to form micelles in which the solubilized drug might have more resistance to diffusion. Thus, the POE(2)LE-containing MCT formulation results in the highest release rate of AMB.

**Intravenous Administration of AMB** Intravenous administration was examined to estimate the absolute bioavailability of AMB for rectal absorption. Figure 2 shows the mean serum concentration of AMB following injection to rabbits. The serum concentration–time data for AMB were analyzed by a two-compartment open model. Pharmacokinetic parameters calculated are presented in Table 2. The \(t_{1/2,\beta}\) was 8.82 ± 0.17 h, indicating rapid elimination of the drug in rabbits in comparison with other animals like dog, monkey, rat and mouse which had \(t_{1/2,\beta}\) of 46.8, 35.3, 16.0 and 27.5 h, respectively.\(^6\)

**Serum Concentration of AMB after Administration of**

![Figure 1: Release Profiles of AMB from Rectal Formulations without (A) or with (B) Surfactants](image)

![Figure 2: Serum Concentration Changes of AMB after Intravenous Administration in Rabbits](image)

![Table 2: Pharmacokinetic Parameters of AMB after Intravenous Administration](table)

**Rectal Formulations without Surfactant** Figure 3 shows the serum AMB concentration profiles after dosing of rectal formulations containing the lyophilized mixtures of AMB and GLYK without surfactant. The AMB dosage forms rectally administered were a solid macrogol suppository, a suspension in liquid MCT and an aqueous solution. The pharmacokinetic parameters of various rectal administrations are given in Table 3, including that of a Witepsol suppository (from ref. 10).
Table 3. Pharmacokinetic Parameters of AMB after Rectal Administration to Rabbits

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cmax (ng/ml)</th>
<th>Tmax (h)</th>
<th>AUC0→24h (h·ng/ml)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>286.9±21.61</td>
<td>2.5±0.9</td>
<td>3512.7±215.21</td>
<td>3.23±0.201</td>
</tr>
<tr>
<td>2</td>
<td>354.2±38.04</td>
<td>1.7±0.7</td>
<td>5363.3±383.85</td>
<td>4.92±0.350</td>
</tr>
<tr>
<td>3</td>
<td>84.2±11.3</td>
<td>1.7±0.7</td>
<td>919.8±62.9</td>
<td>0.85±0.050</td>
</tr>
<tr>
<td>4</td>
<td>205.9±49.0</td>
<td>1.0±0.0</td>
<td>2743.5±619.21</td>
<td>2.52±0.570</td>
</tr>
<tr>
<td>5</td>
<td>68.1±14.0</td>
<td>1.0±0.0</td>
<td>889.2±53.7</td>
<td>0.82±0.050</td>
</tr>
<tr>
<td>6</td>
<td>36.9±6.6</td>
<td>1.0±0.0</td>
<td>352.5±56.2</td>
<td>0.32±0.050</td>
</tr>
<tr>
<td>7</td>
<td>119.1±8.4</td>
<td>3.0±0.0</td>
<td>1504.6±78.0</td>
<td>1.38±0.070</td>
</tr>
<tr>
<td>88[19]</td>
<td>127.2±11.7</td>
<td>1.7±0.5</td>
<td>1638.2±51.7</td>
<td>1.50±0.060</td>
</tr>
</tbody>
</table>

a) F=(AUC0→24h/50g(mg)*100%

Fig. 4. Serum Concentration Changes of AMB after Rectal Administration of Formulations with Surfactants

AMB: GLYK lyophilized mixture (1:9) ○, MCT; □, aqueous solution; △, macro-
gol (1000/4000=1/4); broken line; Witiposol H-15 from reference 10. Each point re-
presthe mean±S.E. of three or four rabbits.

H. M. Lozner et al.

Serum Concentration of AMB after Administration of Rectal Formulations Containing Surfactant

Figure 4 shows the serum AMB concentration profiles after dosing of rectal formulations in which the lyophilized mixtures of AMB and GLYK were incorporated in Witiposol or MCT base with surfactants. The pharmacokinetic parameters are summarized in Table 3. In this absorption test, the investiga-
tion focused on the influence of POE(2)LE on the AMB absorption by comparison with lecithin (HLB: ca. 4) as a newly added surfactant or SLS since the POE(2)LE exhibited the greatest drug release ability (Fig. 1). The SLS addition to Witiposol base gave a marked decline of serum AMB concentration to nearly half of its original level. Lecithin in an MCT base significantly lowered the AUC0→24h as well to about 26% of its original value. Contrary to these results, the addition of POE(2)LE to Witiposol or an MCT base enhanced the AMB absorption. Of all the formulations investigated, the highest bioavailability was 4.92% from the MCT base containing POE(2)LE. It also demonstrated the highest peak serum concentration of 354.9 ng/ml at 1.7 h and maintained a high serum level exceeding 100 ng/ml for 24 h after administration. These results indicate that lipophilic bases are most favorable for the rectal absorption of the lyophilized AMB, and providing a moderate hydrophilicity to the base is likely a major determinant for the enhancement of drug liberation from base and its subsequent absorption.

The drug can be absorbed after it is released from a suppository. In the five AUC0→24h values from MCT, Witiposol and macrogol formulations (Table 3) were plotted against the corresponding release percent at 120 min, the AMB absorption was well correlated to the drug release showing a linear relationship with r=0.98 (Fig. 5). This suggests that the AMB release is the rate limiting step of the rectal absorption. In our previous paper, the lyophilized mixture of AMB and GLYK (1:9) in Witiposol base brought about 35 times greater absorption in AUC0→24h than that of AMB alone. In this study, the absorption of the 1:9 AMB lyophilized mixture in MCT base with POE(2)LE could be further increased by 3.3 times as much as the above Witiposol base most probably due to the improved dissolution rate.

Some investigators have suggested that maintaining serum levels of AMB above the minimal inhibitory concentration (MIC) of the infecting organism is a minimum requirement for successful therapy. It has been reported that the MIC of most Candida species ranges from 50 to 200 ng/ml. To attain the MIC level in serum and to avoid the serious adverse effects by infusion, very large oral doses exceeding 1 g of AMB have been daily given to patients. Taking into account
the serum concentration sufficient for inhibiting the infecting organism, the rectal administration of the lyophilized AMB in MCT base with or without POE(2)LE may provide a promising therapeutic alternative to infusion or oral administration. Although the AMB rectal absorption in this study was still low showing only 4.92% of bioavailability, it is suggested that the extent of absorption can be further enhanced by appropriate formulation design correlating with the dissolution rate.

REFERENCES