Duration of Absorption-Enhancing Effect of Sodium Octanoate, Sodium Hexanoate or Glyceryl-1-monooctanoate on Rectal Absorption of Gentamicin in Rabbits*1)

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The duration of the absorption-enhancing effect of sodium octanoate (C₈), sodium hexanoate (C₆) and glyceryl-1-monoocctanoate (MO) on the rectal absorption of gentamicin (GM) using the hollow-type suppository was investigated in rabbits. To evaluate the duration of the absorption-enhancing effect by pretreatment (treatment of absorption enhancer before GM administration), suppository I containing each absorption enhancer in the cavity was administered into the rectum. Then suppository II containing GM in the cavity was administered at predetermined times (0.33, 2, 6 and 24 h) after the administration of suppository I. Plasma GM levels obtained by the pretreatment with absorption enhancer were compared with those obtained by the simultaneous administration of GM with absorption enhancer. The AUC and C_max of GM significantly decreased with the pretreatment of C₈ (6 and 24 h), C₆ (2 and 6 h) or MO (6 and 24 h) before rectal GM administration, as compared with the simultaneous administration of GM with C₈, C₆ or MO. A marked decrease in the absorption-enhancing effect of C₈, C₆ and MO on rectal GM absorption was observed by the prolongation of the period between the pretreatment of each absorption enhancer and GM administration. The duration of the absorption-enhancing effect of C₈ was shorter than that of C₆, whereas this duration of MO was similar to that of C₆. The effect of these absorption enhancers disappeared 24 h after the pretreatment. These results suggested that the lowering of the membrane transport barrier function recovered about one day after the administration of C₈ or MO.

Keywords — absorption-enhancing effect duration; rectal absorption enhancement; absorption enhancer; sodium octanoate; sodium hexanoate; glyceryl-1-monoocctanoate; gentamicin; hollow-type suppository; rabbit

Introduction

Medium-chain fatty acids2-3) and glycerides,4-6) which increase the mucosal membrane permeability of poorly absorbed drugs, are promising absorption-enhancing agents (absorption enhancers) for rectal dosage forms containing poorly absorbed drugs. To evaluate the applicability of these compounds, studies concerning the effect of formulation factors on rectal absorption enhancement by medium-chain fatty acids and glycerides are very important. In the previous papers, we reported that the formulation factors involving the concentration and dosage form (powder, solution, etc.) of a fatty acid, sodium octanoate (C₈)7) and a monoglyceride, glyceryl-1-monoocctanoate (MO)8) should not be ignored in the evaluation of the enhancement of rectal absorption of a poorly absorbed antibiotic, gentamicin (GM) using hollow-type suppositories9) in rabbits. To better understand the absorption-enhancing effect of these compounds, it is necessary to elucidate the duration of this effect. Although many attempts have been made to estimate the duration of the absorption-enhancing effect of several drugs, only a few experiments on the duration of the enhancing effect of medium-chain fatty acids or glycerides on rectal absorption of poorly absorbed drugs have been reported.

In this study, the duration of absorption-enhancing effects by C₈, sodium hexanoate (C₆) or MO on rectal GM absorption using the pretreatment method (treatment with absorption enhancer before GM administration) was investigated. For the rectal dosage form, the hollow-

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type suppositories were used in order to minimize the drug release difference between GM and the absorption enhancer from the dosage form.

**Materials and Methods**

**Materials** — Gentamicin (sulfate salt, GM) was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Sodium octanoate (C₈, Wako Pure Chemical Industries, Tokyo, Japan) and sodium hexanoate (C₆, Tokyo Kasei Kogyo, Tokyo, Japan) were used. Glycerol-1-monooctanoate (MO, Poem M-100®) was obtained as a gift from Riken Vitamin Co., Ltd., Tokyo, Japan. A suppository base, Witepsol H-15 (Hüls Troisdorf A. G., Troisdorf, FRG) was used. All reagents used were of analytical grade.

**Preparation of Suppository** — A dose of 60 mg GM and 0.18 mmol of each absorption enhancer (C₈ (30 mg, powder), C₆ (25 mg, powder) or MO (39 mg, solid)) were used. This amount of absorption enhancer was sufficient to increase GM absorption in the rectum.⁷,⁸ Formulations comprising the four types of hollow-type suppositories, containing each absorption enhancer in the cavity (suppository I), containing GM in the cavity (suppository II), containing GM and each absorption enhancer in the cavity (suppository III), and containing GM in the cavity and C₈ (30 mg) in the base material of the suppository (suppository IV) were prepared using Witepsol H-15 by the fusion-process method, as described in our previous report.⁹

**Rectal Administration and Determination of Plasma GM Concentration** — Male albino rabbits weighing 2.8—3.2 kg were fasted for one night prior to each experiment. The animals were secured in a crouching posture according to the method described in a previous paper.⁹ The animals were allowed to rest for two weeks before use in the following experiment.

**Pretreatment Method:** Each absorption enhancer (suppository I) was administered into the rectum, then GM (suppository II) was administered 0.33, 2, 6 or 24 h after absorption enhancer administration.

**Simultaneous Administration Methods:** Suppository III or IV was inserted into the rectum. The anus was closed with a plastic clip to prevent leakage during the experimental period. The longest period with the clip was 10 h. After GM administration blood samples were taken from the auricular vein at predetermined intervals and were centrifuged at 3000 rpm for 15 min. The plasma samples were stored at −30 °C until analysis.

GM concentrations in plasma were determined using an enzyme immunoassay (SLFIA)¹¹ kit (Ames TDA®, Ames Division, Miles Laboratories, Inc., Elkhart, IN, U.S.A.).

**Pharmacokinetic Analysis** — The peak plasma GM concentration (Cₘₐₓ), the peak plasma GM concentration time (tₘₐₓ), and the area under the plasma concentration–time curve (AUC) were obtained from the plasma GM concentration–time curves. AUC₀₉ was calculated according to the trapezoidal rule.¹²

Statistical analysis was performed using the one-way ANOVA and the Dunnett’s tests and the differences were assumed to be significant when p<0.05.

**Fig. 1.** Plasma Concentration of GM Following Rectal Administration of GM after Pretreatment with C₈ in Rabbits

Each point represents the mean ± S. E. (vertical bar) of four experiments. Pretreatment time (h): ○, 0; ●, 0.33; ■, 2; ▲, 6; ♦, 24. Pretreatment time 0 h represents simultaneous administration (suppository III) of GM and C₈. Pretreatment time 0.33—24 h is the time interval between the administration of absorption enhancer (suppository I) and GM (suppository II). Dose: GM, 60 mg; C₈, 0.18 mmol.
Duration of Absorption-Enhancing Effect

Fig. 2. AUC of GM Following Rectal Administration of GM after Pretreatment or Simultaneous Administration of Absorption Enhancer in Rabbits

Each point represents the mean ± S. E. (vertical bar) of four experiments. □, simultaneous administration (suppository III); □, pretreatment (suppository I and II); ■, simultaneous administration (suppository IV). Dose: GM, 60 mg; absorption enhancer 0.18 mmol. Statistically significant differences: a) p<0.01 in 6 and 24 h vs. 0 h, b) p<0.05 in 2 and 6 h vs. 0 h.

Results and Discussion

Rectal GM Absorption with Pretreatment of C₈ or C₆

For the formulation of dosage form, to render easy delivery of GM and absorption enhancers to the rectal lumen, hollow-type suppositories were employed since we had previously observed the rapid release of drug from this prepara-
tion. The dissolution of GM and absorption enhancer is more rapid with the hollow-type suppository than with the conventional suppository.

The plasma concentration—time curves after GM administration with the C₈ pretreatment are shown in Fig. 1. The AUC₀₋₄ of GM is shown in Fig. 2, and the values of Cₘₐₓ and tₘₐₓ are shown in Table I.

Without an absorption enhancer, GM was not absorbed (plasma GM levels less than 1 μg/ml) from the rectum. However, GM was significantly absorbed after simultaneous administration with C₈, and the highest plasma GM level of 21.5 ± 3.1 μg/ml (Cₘₐₓ) was obtained (Fig. 1). A marked decrease of plasma GM concentration was observed when the time interval between the pretreatment with C₈ and the administration of GM was prolonged. The values of Cₘₐₓ and AUC obtained by the pretreatment with C₈ at 6 and 24 h were statistically lower (p<0.01, p<0.05) than those obtained by the administration of GM and C₈.

Plasma GM concentration after simultaneous administration with C₆ was lower than that by the use of C₈. This result shows that the absorption-enhancing effect of C₆ was less than that of C₈. The values of Cₘₐₓ (Table I) and AUC (Fig. 2) decreased significantly after the pretreatment with C₆. The absorption-enhancing effect disappeared 2 h after C₆ administration. The absorption-enhancing effect of C₆ was much reduced from that of C₈. This result is probably related to the difference in the absorption-enhancing potencies of the two com-

Table I. Cₘₐₓ and tₘₐₓ of GM Obtained after Administration of GM and Absorption Enhancer in Rabbits

<table>
<thead>
<tr>
<th>Pretreatment time³⁷ (h)</th>
<th>C₈</th>
<th>C₆</th>
<th>MO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cₘₐₓ (μg/ml)</td>
<td>tₘₐₓ (min)</td>
<td>Cₘₐₓ (μg/ml)</td>
</tr>
<tr>
<td>0³⁷</td>
<td>21.5 ± 3.1</td>
<td>30 ± 0</td>
<td>12.3 ± 0.7</td>
</tr>
<tr>
<td>0.33</td>
<td>17.8 ± 2.4</td>
<td>23 ± 4</td>
<td>7.0 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>13.0 ± 1.2</td>
<td>52 ± 8</td>
<td>3.8 ± 0.3³⁷</td>
</tr>
<tr>
<td>6</td>
<td>5.8 ± 1.9³⁷</td>
<td>75 ± 36</td>
<td>2.8 ± 0.9³⁷</td>
</tr>
<tr>
<td>24</td>
<td>1.7 ± 0.3³⁷</td>
<td>110 ± 10</td>
<td>—</td>
</tr>
</tbody>
</table>

a) Pretreatment time 0.33—24 h is the time interval between the administration of absorption enhancer (suppository I) and GM (suppository II). b) Pretreatment time 0 h represents simultaneous administration (suppository III) of GM and absorption enhancer. Statistically significant differences: c) p<0.05 in 2 h vs. 0 h. d) p<0.01 in 6 h vs. 0 h. e) p<0.05 in 6 h vs. 0 h. f) p<0.01 in 2 4 h vs. 0 h. g) p<0.05 in 2 4 h vs. 0 h.
Rectal GM Absorption with MO Pretreatment

The plasma GM concentration–time curves obtained after administration of suppository I and II or III are illustrated in Fig. 3. The $AUC$ values are summarized as histograms in Fig. 2. The values of $C_{\text{max}}$ and $t_{\text{max}}$ are summarized in Table I.

The value of $AUC$ obtained by the simultaneous administration of GM with MO was nearly equal to that obtained by the simultaneous administration of GM with C$_8$ (Fig. 2). Thus, the absorption-enhancing effect of MO was approximately equivalent to that of C$_6$. However, in comparing the value of $AUC$ observed after the pretreatment (2 h) of C$_8$ with MO, the $AUC$ obtained by MO pretreatment (2 h) was significantly higher than that by C$_6$ pretreatment (2 h). The absorption-enhancing effect of MO on GM absorption disappeared 24 h after pretreatment.

Concerning the effect of pretreatment with fatty acid and glyceride on poorly absorbed drugs, oleic acid in the micellar state and medium-chain glyceride mixture (MGK) in emulsion have been investigated. The absorption-enhancing effect of these materials rapidly disappeared and became undetectable in a short time (within 60 min) after the treatment. The recovery of membrane barrier function was achieved within 1 h. However, our results in this study indicate that the absorption-enhancing effects of C$_8$ and MO remained for several hours. This difference in the duration of the enhancing effect is probably related to the difference in concentration of absorption enhancers in the rectum because of the powdered form administered. Concerning the mechanism of action of C$_8$ and MO, absorption of these compounds could not be confirmed because of very low plasma C$_8$ levels and esterification. According to the reports of McNeil et al., Pihl et al. and Sekine et al., it is presumed that either compound could be slightly absorbed. Therefore, the absorption-enhancing effect remained for several hours. With respect to the difference of the duration of enhancing effect between absorption enhancers, they may remain on the rectal lumen and play an essential role in the absorption process. Although the amount of absorption en-
hancer on the rectal lumen is important, we could not determine these residues because of interference with suppository base materials.

Plasma Concentration of GM after Rectal Administration of Suppositories III and IV

The \( t_{\text{max}} \) of GM obtained by the rectal administration 20 min after pretreatment with each absorption enhancer was shorter than that obtained by the simultaneous administration of GM and absorption enhancer (Table I). The result for MO suggests that the rate of GM bioavailability of suppository II administered 20 min after pretreatment with MO is higher than that of suppository III. To elucidate the cause of this difference in the rate of GM bioavailability, the absorption of GM with suppository IV was compared with that of suppository III. In the case of a short interval (20 min) between the administration of the absorption enhancer and GM, the amount of the suppository base (Witepsol H-15) may influence the effect of the absorption enhancer. However, this influence may be ignored in evaluating the duration of the enhancing effect because there was no difference in the \( AUC \) values obtained (Fig. 2).

The plasma GM concentration–time curves are shown in Fig. 4. The values of \( AUC \) are summarized as the closed column in Fig. 2. By the use of suppository IV, plasma GM levels 15 and 30 min after administration were significantly higher than those of suppository III. Although the \( AUC \) and \( C_{\text{max}} \) were not different between the two suppositories, the mean value of \( t_{\text{max}} \) of suppository IV (ca. 13 min) was less than those of suppository III (30 min). It is presumed that the absorption enhancer can reach the rectal lumen from the base material of suppository IV before the release of GM in the hollow cavity. Consequently, GM was rapidly absorbed when the absorption enhancer was delivered prior to GM administration. Previously, we observed a similar result wherein the \( t_{\text{max}} \) obtained after the administration of the suppository containing GM in the cavity and MO in the body was less than that from the suppository containing GM and MO in the cavity.\( ^8 \)

Conclusions

GM could be absorbed even when the enhancer was administered about 2 h before GM administration. However, the extent of GM absorption significantly decreased with the prolongation of the period between pretreatment and administration of GM. Consequently, GM was scarcely absorbed 24 h after the pretreatment of absorption enhancer. Although the duration of the absorption enhancing effect depends on the amount of absorption enhancer, the lowering of the membrane transport barrier function in the rectum recovered about one day after the administration of at least 0.18 mmol of absorption enhancer tested in this investigation. Especially, when weak potency or a smaller amount of absorption enhancer is used, the enhancing effect varied with the release of absorption enhancer from the suppository.

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