Rectal Absorption of Acyclovir in Rats and Improvement of Absorption by Triglyceride Base

Taro Ogiso,* Masahiro Iwaki, Tadatoshi Tanino, Junji Fuhi and Tsuyoshi Paku

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1, Kowakae, Higashi-Osaka, Osaka 577, Japan.
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The rectal absorption of acyclovir has been evaluated after administration of suppositories without absorption enhancers in rats. The disappearance of plasma acyclovir followed biexponential kinetics after i.v. dosing. Rectal administration of a triglyceride (Vosco S-55 and Vosco S-55 + methylcellulose) suppository gave relatively high plasma concentrations and bioavailabilities (95.3 and 83.4%, respectively) compared with Witapsol and macrogol suppositories. However, the in vitro release profiles from suppositories did not accurately reflect plasma concentrations after rectal dosing. Our results suggest that the rectal administration of acyclovir suppositories may be a promising substitute for intravenous infusion, which is at present used for the treatment.

Keywords acyclovir; rectal administration; rat; high bioavailability; in vitro release; suppository

Acyclovir, 9-(2-hydroxyethoxymethyl)guanine, a potent and selective antiviral agent, is poorly and slowly absorbed from the gastrointestinal tract of man. Brigiden et al. reported that the percentage of the dose recovered in urine after oral administration of acyclovir was 6—13.2%. The estimated total bioavailability of acyclovir in man is between 15 and 30% and it appears that the bioavailability decreases with increasing doses. To enhance the absorption of acyclovir, rectal administration in the presence of sodium salts of fatty acids was designed in rats, and resulted in enhanced bioavailability (81 ± 3%). At present, acyclovir is available in topical and intravenous formulations, although an oral tablet of acyclovir has recently been marketed. Practically, acyclovir is given as a continuous infusion of daily doses. However, this drug treatment will not be convenient to child patients with herpes virus infections. In this study, we investigated the effect of suppository bases on the rectal absorption of acyclovir without enhancers. For a trial use of the suppository as an alternative to injection, we administered the suppositories once a day for 2 d to the rats, and the plasma levels of acyclovir were determined. The plasma concentrations obtained after rectal administration were discussed in relation to the antiviral activity against herpes viruses.

Materials and Methods

Materials Acyclovir was supplied by Nippon Wellcome Co. (Osaka). Adenosine, an internal standard for high performance liquid chromatography (HPLC), was purchased from Sigma Chemical Co. (St. Louis, MO). Suppository bases, Witapsol H-15, macrogol (1500 and 4000) and Vosco S-55 were obtained from Mitsuba Boeki Co., Nacalai Tesque Co. and Maruishi Pharmaceutical Co., respectively. All other chemicals were of analytical or special grade. Male Wistar rats weighing 200—260 g were used throughout this experiment. The animals, maintained on a MF diet (Oriental Yeast Co.) for 3—4 d prior to the experiment, were divided at random into 3—4 groups, each consisting of 4—7 rats. On the day before the experiment, the jugular vein was cannulated with silicon tubing.

Preparation of Suppository The suppositories were prepared by the fusion method at a temperature as low as possible, using Witapsol H-15, macrogol (4000:1500 = 3:1, w/w) or Vosco S-55 as bases. One cm length of the suppository (diameter 4 mm) contained 10 mg acyclovir. Details of the composition are listed in Table 1.

Intravenous (i.v.) Administration Acyclovir, dissolved in 150 μl NaOH and diluted with 0.1 M phosphate buffer (pH 7.0), was administered intravenously through the tubing at a dose of 25 mg/kg. After administration, blood samples were withdrawn from the cannulated jugular vein periodically into a heparinized syringe. The plasma was separated immediately by centrifugation and stored frozen until assay.

In Vitro Rectal Absorption Experiment The animals were fasted for 24 h before and during the experiment of rectal administration. The suppository was inserted to a depth of 1 cm from the anus, and the anus was closed with an adhesive, Aronalpha (Konishi Co.). The plasma obtained was stored frozen until assay. In some cases, a Vosco S-55 suppository (acyclovir, 40 mg/kg) was administered once a day for 2 d.

Determination of Acyclovir Acyclovir in plasma and the sample was determined by the method of Krasny et al. using adenosine as an internal standard, with slight modifications. A 100 μl aliquot of plasma was mixed with 10 μl of adenosine (250 μg/ml methanol) and 190 μl of methanol. Following centrifugation, the supernatant was filtered through a membrane filter (0.45 μm, Cosmonice W, Japan Milipore Industrial Co.). The filtrate was injected into a reverse-phase COSMOSIL SC-8 column (5 μm, 4.6 × 150 mm, Nacalai Tesque). Two % ethanol in 0.05 M KH₂PO₄ buffer (pH 3.3) was used as the mobile phase. The limit of detection of acyclovir in plasma was approximately 0.7 μg/ml.

In Vitro Release of Acyclovir from Suppositories The release rates of acyclovir were measured by the method of Muranishi et al., using a cylinder filter paper and 0.067 mm phosphate buffer, pH 7.3, (50 ml) at 37°C.

Analysis of Data Kinetic parameters were calculated by using the least squares fit program, MULTI. The plasma concentration data after i.v. administration were calculated by the following equation:

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

where \( C_t \) is the drug concentration at time \( t \) and \( A, \alpha, B, \beta \) are the biexponential equation constants.

To analyze the plasma concentration after rectal dosing, the data were fitted to the 1-compartment model including a first order absorption process, and parameters were determined by the fitting:

\[ C_t = \frac{F \cdot D \cdot k_d}{V_1 (k_e - k_a)} (e^{-k_e t} - e^{-k_a t}) \]

where \( k_a \) is the apparent absorption rate constant, \( F \) is the fraction of drug absorbed, \( V_1 \) is the apparent distribution volume and \( D \) is the dose of drug.

**Table 1. Composition of Acyclovir (ACV) Suppositories**

<table>
<thead>
<tr>
<th>No.</th>
<th>ACV (mg)</th>
<th>Witapsol H-15 (mg)</th>
<th>Macrogol 4000:1500 (mg)</th>
<th>Vosco S-55 (mg)</th>
<th>MC (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>110</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>—</td>
<td>105:35</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>110</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

MC, methylcellulose.

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The AUC after i.v. and rectal dosings was determined by the trapezoidal rule to the last observed data point. The absolute bioavailability was calculated using the AUC values.

The area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by means of the following equations:

\[ AUMC = \int_0^\infty t \cdot Cdt \]
\[ MRT = \frac{AUMC}{AUC} \]

The means of all data are presented with their standard deviation (mean ± S.D.). Statistical analysis was performed by using the non-paired Student's t-test, and the significance level adopted was \( p < 0.05 \).

**Results and Discussion**

**Plasma Concentration of Acyclovir after Single i.v. Administration**

The plasma concentrations after a single i.v. administration of acyclovir (25 mg/kg) are shown in Fig. 1. Since the plasma decay curve after dosing showed bi-exponential kinetics, it was analyzed by the 2-compartment open model. Pharmacokinetic parameters calculated by using the data are listed in Table II. The \( t_{1/2\beta} \) and MRT were 1.78 h\(^{-1}\) and 1.33 h, respectively, indicating the rapid elimination of the drug in rats. The \( t_{1/2\beta} \) value agreed with the data (1.39—2.67 h in rat) reported by another study.\(^9\)

**Plasma Concentration of Acyclovir after Single Rectal Administration of Suppositories**

There has been renewed interest in rectal administration of acyclovir as a possible way to administer the drug and improve the intravenous infusion therapy which is restricted by time.

The plasma acyclovir concentration profiles and kinetic parameters after dosing of Witensol H-15 and macrogol suppositories are shown in Fig. 2A and Table III, respectively. The plasma concentrations after Witensol H-15 suppository were slightly higher at the initial time stage than those after macrogol suppository, but the plasma elimination of acyclovir was more rapid after Witensol H-15 suppository and the plasma concentration was not detected 10 h after dosing. The bioavailabilities after administration of Witensol and macrogol suppositories were 49.5 and 49.4\%, respectively. These values roughly agreed with the data (37 and 20\%, respectively) reported by Yamazaki et al. However, we could determine the plasma levels for a much longer time compared with their sampling time (for 3 h) because our method could detect as low a concentration of acyclovir as 0.7 \( \mu \)g/ml plasma by using an internal standard.

![Graph A](image1)

**Fig. 1.** Plasma Acyclovir Concentration after i.v. Administration

Each point represents the mean ± S.D. (\( n = 4—7 \)). The dose was 25 mg/kg. The solid line represents a simulation curve.

**Table II.** Pharmacokinetic Parameters after Intravenous Administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (µg/ml)</td>
<td>63.18 ± 17.83</td>
</tr>
<tr>
<td>α (h(^{-1}))</td>
<td>2.39 ± 1.93</td>
</tr>
<tr>
<td>B (µg/ml)</td>
<td>7.83 ± 5.37</td>
</tr>
<tr>
<td>β (h(^{-1}))</td>
<td>0.39 ± 0.29</td>
</tr>
<tr>
<td>( k_{12} ) (h(^{-1}))</td>
<td>0.64 ± 0.24</td>
</tr>
<tr>
<td>( k_{21} ) (h(^{-1}))</td>
<td>0.61 ± 0.19</td>
</tr>
<tr>
<td>( k_{e} ) (h(^{-1}))</td>
<td>1.54 ± 0.32</td>
</tr>
<tr>
<td>AUMC (µg·h/ml)</td>
<td>47.25 ± 16.38</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.33 ± 0.46</td>
</tr>
</tbody>
</table>

\( k_{12} \) and \( k_{21} \) are the distribution rate constants and \( k_{e} \) is the elimination rate constant. Each value represents the mean ± S.D. (\( n = 4—7 \)).

![Graph B](image2)

**Fig. 2.** Plasma Acyclovir Concentration after Rectal Administration

Each point represents the mean ± S.D. (\( n = 3—5 \)). The dose was 40 mg/kg. (A) ○, macrogol 4000:1500 (3:1, w/w); ●, Witensol H-15; (B) ◊, Vosco S-55; ■, Vosco S-55 + MC. \( * p < 0.05 \) compared with the other suppository.

**Table III.** Pharmacokinetic Parameters of Acyclovir after Rectal Administration

<table>
<thead>
<tr>
<th>Base</th>
<th>( C_{max} ) (µg/ml)</th>
<th>( AUC_{0–10} ) (µg·h/ml)</th>
<th>Bioavailability (%).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witensol H-15</td>
<td>11.45 ± 3.71</td>
<td>37.43 ± 5.51</td>
<td>49.5 ± 10.1( * )</td>
</tr>
<tr>
<td>Macrogol 4000:1500</td>
<td>7.92 ± 1.35</td>
<td>37.37 ± 3.48</td>
<td>49.4 ± 4.4</td>
</tr>
<tr>
<td>(3:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vosco S-55</td>
<td>24.02 ± 7.64( * )</td>
<td>72.03 ± 12.19( * )</td>
<td>95.3 ± 16.0( * )</td>
</tr>
<tr>
<td>Vosco S-55 + MC</td>
<td>29.53 ± 5.53( * )</td>
<td>63.03 ± 3.49( * )</td>
<td>83.4 ± 4.0( * )</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (\( n = 3—5 \)). Dose, 40 mg/kg. \( * p < 0.05 \) compared with Witensol H-15.
The plasma levels after the administration of Vosco S-55 and Vosco S-55 plus methylcellulose (MC) suppositories are shown in Fig. 2B. The plasma levels after the administration of both suppositories were higher than those after Witepsol and macrogol suppositories. The maximum plasma level (C_{max}) after dosing of Vosco S-55 plus MC suppository was the highest of these suppositories, as shown in Table III. The bioavailability of acyclovir after Vosco S-55 and Vosco S-55 plus MC suppositories thus was considerably high (95.3 ± 16.0 and 83.4 ± 4.0%, respectively). The increased absorption after Vosco S-55 suppository may be owing to the enhanced transport through the mucosal membrane by the action of mono- and diglycerides. The difference between the parameters obtained with both Vosco S-55 and Witepsol H-15 suppositories may be due to the difference in base composition.\(^\text{10}\) With the intention of suppressing the rise of plasma concentration at the initial time stage and sustaining the plasma level for a long time, MC was added to the Vosco S-55 base. However, the objective effect was not obtained by the additive. This may be due to the fact that MC partly enhanced the release rate of the drug from the suppository by swelling based on absorbing water, judging from the results of release test.

The gastrointestinal absorption mechanism of acyclovir is predominantly via passive diffusion.\(^\text{9}\) Therefore, the absorption through rectal mucosa might also be due to passive diffusion.

**Plasma Concentration of Acyclovir after Repeated Rectal Administration of Vosco S-55 Suppository** Since the results obtained from the single dosing indicated that acyclovir was better absorbed from a Vosco S-55 suppository, this suppository was administered once a day for 3d. However, since on the third day the rat's strength was weakened by repeated fasting, blood sampling was not carried out. The plasma acyclovir concentration profile and the kinetic parameters (day 1 and 2, C_{max}, 25.10 and 26.17 μg/ml; AUC_{0-24hrs}, 64.13 and 61.68 μg·h/ml; t_{1/2}, 0.250 and 0.331 h⁻¹, respectively) for 2d were similar to each other.

To examine the possibility of an alternative to intravenous infusion, we administered a Vosco S-55 suppository to rat once a day for 2d. The C_{max} was 24 μg/ml and the plasma levels above 2.0 μg/ml were maintained for 10h. The level required to inhibit the replication of herpes viruses by 50% (ED_{50}) are shown in Table IV.\(^\text{11,12}\) The plasma concentration of acyclovir after rectal administration were generally higher than the ED_{50}. Although there are species differences among human and rat, the higher plasma levels obtained suggest that therapy using the acyclovir suppository against infections from herpes viruses may be a promising substitute for the infusion method. One benefit of using the suppository is the possibility of administering the dosage at bed time for child patients, without restriction.

**In Vitro Release of Acyclovir from Suppositories** To estimate the effect of drug release on rectal absorption, the release from suppositories into the buffer at pH 7.3 was studied. The result is shown in Fig. 3. When the data for the concentration of acyclovir released were plotted versus h^{1/2}, the resulting curve approximated a straight line, except for the data of the Vosco S-55 plus MC suppository. The release profile from this suppository consisted of two distinct slopes, a slow release at the first time point and then a comparative rapid release.

As a result, the release profiles (Table V) of acyclovir from the suppositories did not accurately reflect the plasma concentration after dosing, suggesting that the release rate is not a rate limiting step of the absorption.

In conclusion, Vosco S-55 and Vosco S-55 plus MC suppositories of acyclovir gave relatively high plasma concentrations after administration. The single and repeated administrations of these suppositories resulted in effective plasma concentrations sufficient for inhibiting herpes viruses. Consequently, the suppositories make it possible to treat infections caused by herpes viruses.

**References**


