Preparation and Rectal Absorption of Highly Concentrated Glycyrrhizin Solution

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We developed a simple method for preparing a highly concentrated solution of glycyrrhizin monoammonium salt (GZ) at low viscosity with no surfactants nor organic solvents and investigated the absorption profile after rectal administration to rats. GZ (200 mg/ml) was dissolved in phosphate buffered solution, pH 7.0; over 350 mM concentration was maintained for the aqueous solution without gel-formation. When glycerin was used as a non-aqueous formulation, GZ did not form gel. Apparent permeability coefficients of GZ obtained from 350 mM phosphate buffered solution (pH 7.0) and glycerin solution through rat rectal mucosa estimated by in vitro parallel diffusion chamber technique were $0.686 \times 10^{-6}$ and $0.379 \times 10^{-6}$ cm/s, respectively. On the other hand, the area under plasma concentration–time curves of GZ in 400 mM phosphate buffer (pH 7.0) and glycerin formulations after rectal administration to the rat were significantly higher than that in polyethylene glycol 400/propylene glycol (55:5) formulation. Maximum plasma concentrations of these formulations were dependent on the apparent permeability coefficients of GZ. Increased absorption observed by phosphate buffered formulation accompanied no pronounced histological damage in mucosa. These results demonstrate that addition of a highly concentrated phosphate salts is effective not only for lowering the viscosity of a highly concentrated solution of GZ solution, but also for improving the mucosal GZ absorption.

Key words glycyrrhizin; gel-formation; phosphate buffered solution; rectal absorption; rat

Glycyrrhiza glabra L., is the glycoside of glycyrrhetic acid. GZ shows antiviral activity, interferon inducing activity, inhibition of HIV-1 replication, etc. In particular, it has been reported that high dose intravenous (i.v.) administration of GZ (200 mg/kg) is effective for treatment of chronic hepatitis. However, oral administration of GZ can not elicit effectiveness equal to i.v. administration because GZ is hydrolyzed in a stepwise manner to the monoglucuronide and then to glycyrrhetic acid through action of endogeneous (biliary and enteric) and bacterial $\beta$-glucuronidases in the intestinal lumen. When GZ (100 mg) was orally administered to men, GZ was not detected in serum. In contrast, GZ was detectable in plasma following oral administration of a large dose (500 mg/kg) to rats. If a large dose of GZ is formulated into a pharmaceutical preparation, there is a possibility that GZ is absorbed into the systemic circulation and therapeutically available amount of GZ is detected in plasma after oral or other different route of administration aside from i.v. administration. Physicochemical properties of GZ include its free solubility in dilute ethanol and slight solubility in water. Furthermore, although GZ dissolves in phosphate buffered solution at a higher temperature (> approximately 60 °C), GZ easily forms a gel at room temperature. The aim of this study was to develop a simple method for preparation of a concentrated GZ formulation (200 mg/ml as GZ concentration) by avoiding its gel-formation and to investigate the bioavailability of GZ after rectal administration to rats to estimate GZ usefulness.

MATERIALS AND METHODS

Chemicals GZ monoammonium salt (GZ) was received from Koki Co. (Tokyo, Japan). Glycerin, polyethylene glycol 200 (PEG 200), polyethylene glycol 400 (PEG 400), propylene glycol (PG) and triacetin were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other materials were of reagent grade.

Animals Male Wistar rats (220—280 g) were fed usual laboratory chow and had free access to water. They were fasted for 16 h before use. All animals received human care and were treated in accordance with Guidelines for Animal Experimentation at Hokuriku University.

Solubility of Glycyrrhizin After 200 mg GZ was added to a vial with 5 ml of various concentrations of ethanol or 100 mM phosphate buffered solution with various pH values, all samples were shaken for 30 min at 300 rpm using a Vortex shaker (VR-36, Taiiec, Koshigaya, Japan) at room temperature. After the GZ solution was filtered with a Millipore filter.

Fig. 1. Structure of Glycyrrhizin

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Preparation of GZ using non-aqueous excipients such as PG, glycerin, PEG 200, PEG 400 and triacetin was as follows: 1.5 g GZ and 5 ml of each excipient were shaken for 24 h at 500 rpm using a Vortex shaker at 25 °C. After each excipient with GZ was filtered with a Millipore filter (LCR13-LH), the GZ concentration was measured by an HPLC.

**Thermotropic behavior and Gel-Forming Temperature of Glycyrrhizin** For the synergistic effect of ethanol concentration and pH on the GZ solubility, 1.0 g GZ was dissolved with 5 ml of 100 mM phosphate buffered solution (pH 9.0) with 10—50% ethanol contents at 60 °C. The temperature was decreased at the rate of 2 °C per 10 min. GZ conditions were observed as thermotropic behavior at the range of 60 to 20 °C.

For the assay of gel-forming temperature of GZ, after 1.0 g GZ was added to a vial with 5 ml of various concentrations of phosphate buffer (100—400 mM) adjusted to pH 7.0 or pH 8.0, all samples were heated at 60 °C in a water bath. The temperature was decreased at the rate of 2 °C per 10 min up to 10 °C. Gel-forming temperature of GZ was decided in the start of gel-formation.

**Preparation of Glycyrrhizin Formulation** GZ was dissolved with aqueous or non-aqueous solvents i.e., 400 mM phosphate buffered solution (pH7.0), glycerin and PEG 400/PG (55 : 5, v/v), using a hotplate stirrer (DP-1L, Asone, Tokyo, Japan) for 30—120 min at 60 °C to make each formulation clear. The GZ concentrations in three formulations for in vivo administration clear. The GZ concentrations in three formulations for in vivo administration of GZ 50 mg/kg during the experiment.

**In Vitro Permeability Study** Rats were killed by removing blood from the main abdominal artery after intraperitoneal administration of sodium pentobarbital 50 mg/kg. A ca. 4 cm length of the jejunum or colon was excised immediately with scissors. After the tissue segment was spread to a flat sheet with scissors, the tissue segment sheet was set between Ussing-type diffusion chambers (No. 3440-s, Navicyte Inc., NV, U.S.A.); then, mucus was removed with Dulbecco’s phosphate buffered saline.

100 mM phosphate buffered solution adjusted to pH 7.0 was used as the buffer for the serosal side chamber. 5 mM GZ dissolved with 100 mM phosphate buffered solutions (pH 6, 7 and 8) or non-aqueous excipients was included in the mucosal side chamber. Total volume of each chamber was fixed at 3 ml. During experiment, both chambers were incubated at 37 °C and bubbled with O₂/CO₂ (95 : 5, v/v) gas. A 100 μl sample was taken from the serosal side at 30, 45 and 60 min of incubation; then samples were filtered with a Millipore filter (LCR13-LH). Finally, 30 μl volume was injected into the HPLC system.

**Apparent Permeability Coefficient** The permeation rate of GZ was expressed as an apparent permeability coefficient (P_app) according to the following equation:

\[
P_{\text{app}} = \frac{dC}{dt} V C_0 A
\]

where \(dC/dt\) is the change in concentration per unit time (nmol/ml/s), \(V\) is the solution volume of the receptor side (3 ml), \(C_0\) is initial drug concentration of the donor side (nmol/ml), and \(A\) is apparent surface area of the jejunum sheet (1.2 cm²).

**In Vivo Absorption Study** Rats were kept in an anesthetized condition by intraperitoneal administration of sodium pentobarbital 50 mg/kg during the experiment. GZ formulation was administered to the rectum using an anus sonde (50 mg/0.5 ml/kg). Immediately the anus was closed using a suture material in order to prevent the leak of test formulation. Blood (200 μl) was collected from the tail vein at predetermined times. After plasma was separated from blood by centrifugation at 1680×g for 15 min, GZ concentration in the plasma sample was determined by the HPLC.

**Pharmacokinetic Analysis** Plasma concentration vs. time curves of GZ after rectal administration were fitted using a non-linear least-squares program MULTI. The pharmacokinetic parameters were calculated based on the 2-compartment open model:

\[
C_t = A \exp(-\alpha t) + B \exp(-\beta t) - C \exp(-k_t t)
\]

where \(C_t\) is plasma GZ concentration at time \(t\), \(A, B\) and \(C\) are ordinate intercepts, \(\alpha\) and \(\beta\) are the corresponding first-order disposition rate constants and \(k_t\) is the first-order absorption rate constant. Elimination half-life \((t_{1/2}\beta)\) was calculated by dividing \(\ln 2\) by \(\beta\). CL_renal was calculated by dose (50 mg/kg)/AUC. AUC was calculated by the trapezoidal rule and extrapolated to the infinity. Bioavailability (BA) of GZ was determined by the ratio of AUC after rectal administration to that after i.v. administration cited from the reference; plasma GZ concentration vs. time data after i.v. administration of GZ 50 mg/kg dose to rats.

Changes in the absorption rate of GZ after rectal administration of GZ formulations were estimated by deconvolution method from plasma GZ concentration vs. time data obtained after i.v. and rectal administrations.

**Morphologic Observation** At the end of in vivo absorption study, the bottom, ca. 2 cm length, of the large intestine was excised using scissors. The tissue was sectioned into three segments. Each segment was pretreated with tissue fixation liquid (4% paraformaldehyde and 1% glutaraldehyde) for 4 h; then each sample was dehydrated with 50, 70, 90, 95 and 100% ethanol by processing every 1 h. All tissue segments were solidified by adding a small amount of mixture of hydroxypropyl methyl metacrylate/Quetol-523/methyl metacrylate (65 : 10 : 25) containing 1 mg/ml QC-U1 for 20 h at 60 °C. Slices were dyed in hematoxylin and eosin after tissues were cut using a glass knife.

**Assay of Glycyrrhizin** The GZ concentration in vivo and in vitro samples was determined according to the HPLC method previously reported. In brief, the HPLC condition was as follows: the column was a Nucleosil 5C18 (4.6 mm i.d., 250 mm length); the column temperature was maintained at 40 °C for all separation; detection was performed at UV-wavelength of 254 nm; the mobile phase was methanol/distilled water/25% ammonia solution/60% perchloric acid (53 : 47 : 0.5 : 0.5, v/v); and the flow rate of mobile phase was 0.8 ml/min. The GZ concentration in plasma was assayed after extraction with methanol. For the quantitative determination of GZ, calibration curve was obtained with indomethacin as an internal standard. The correlation coefficients for the calibration curves were 0.999 or better, and the detection limit was 0.2 μg/ml.

**Statistics** All the values are shown as the mean±S.D.
RESULTS

**Glycyrrhizin Solubility in Aqueous Solution**  
Figure 2A shows GZ solubility in various ethanol concentrations. The GZ solubility did not show a linear relationship with ethanol contents, which showed biphasic pattern with the maximum GZ solubility (26.8 mg/ml) at 30% ethanol content. GZ solubility was 25—27 mg/ml at the range of 30 to 45% ethanol, and was decreased by increasing ethanol concentration at the range of 45 to 60%.

Secondly, the effect of pH on GZ solubility was studied. The GZ solubility was dependent on pH (Fig. 2B). The GZ change from a solution with turbidity to a clear solution was dependent the value of pH. When 100 mM phosphate buffered solution (pH 8.9) was used, the GZ solubility reached 40 mg/ml. Based on the results that GZ solubility was improved in the conditions of 30—45% ethanol and high pH values, the synergistic effect on GZ solubility was examined in the condition of pH 9.0 and 10—50% ethanol concentrations. Table 1 shows the relationship between temperature and thermotropic behavior of GZ prepared with various ethanol concentrations in 100 mM phosphate buffered solution (pH 9.0). The addition of ethanol at pH 9.0 remarkably increased GZ solubility. Namely, the clear solution of 200 mg/ml GZ was obtained with the existence of 30—40% ethanol at 30 °C. However, GZ changed to a pale white solution with turbidity at 20 °C.

**Relationship between Buffer Concentration and Gel-Forming Temperature of Glycyrrhizin Solution**  
Results showed that preparation of a 200 mg/ml GZ solution was difficult at 20 °C. Therefore, we studied whether increased phosphate buffer concentration improved GZ solubility. Figure 3 shows the relationship between pH 7.0 or pH 8.0 phosphate buffer concentration and gel-forming temperature of GZ. Gel-forming temperatures in both pH solutions decreased with phosphate buffer concentration increased: a good correlation was observed between both parameters. Actually, when phosphate buffered solutions of 350 mM for pH
Table 2. Solubility of Glycyrrhizin (GZ) by Addition of Various Excipients

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene glycol</td>
<td>111±9.58</td>
</tr>
<tr>
<td>Glycerin</td>
<td>243±4.89</td>
</tr>
<tr>
<td>PEG 200</td>
<td>188±2.77</td>
</tr>
<tr>
<td>PEG 400</td>
<td>164±2.10</td>
</tr>
<tr>
<td>Triacetin</td>
<td>1.23±0.07</td>
</tr>
</tbody>
</table>

1.5 g of GZ and 5 ml of each excipient were shaken for 24 h at 500 rpm at 25°C. After supernatants were filtered, GZ concentrations were measured by an HPLC. Data represent the mean±S.D. of three experiments.

Table 3. Effect of pH on $P_{app}$ Value of Glycyrrhizin (GZ) in Rat Colon

<table>
<thead>
<tr>
<th>pH of mucosal side</th>
<th>$P_{app} (\times 10^{-6} \text{ cm/s})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>0.112±0.065</td>
</tr>
<tr>
<td>7.0</td>
<td>0.686±0.131</td>
</tr>
<tr>
<td>8.0</td>
<td>1.057±0.166</td>
</tr>
</tbody>
</table>

The assay of $P_{app}$ value was carried out using Ussing-type diffusion chambers at 37°C. 100 m M phosphate buffered solution (pH 6.0, 7.0 or 8.0) with 5 m M GZ was used in the mucosal side. 100 m M phosphate buffered solution (pH 7.0) was used in the serosal side. Data represent the mean±S.D. of three experiments.

Table 4. $P_{app}$ Values of Glycyrrhizin (GZ) Dissolved in Various Excipients in Rat Colon

<table>
<thead>
<tr>
<th>Excipients</th>
<th>$P_{app} (\times 10^{-6} \text{ cm/s})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>0.379±0.064</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>0.019±0.005</td>
</tr>
<tr>
<td>PEG 200</td>
<td>0.048±0.008</td>
</tr>
<tr>
<td>PEG 400</td>
<td>0.017±0.005</td>
</tr>
<tr>
<td>G · PEG 400 (90 : 10, v/v)</td>
<td>0.186±0.030</td>
</tr>
<tr>
<td>G · PEG 400 (70 : 30, v/v)</td>
<td>0.082±0.017</td>
</tr>
<tr>
<td>G · PEG 400 (50 : 50, v/v)</td>
<td>0.077±0.011</td>
</tr>
</tbody>
</table>

The assay of $P_{app}$ value was carried out using Ussing-type diffusion chambers at 37°C. Non-aqueous excipients with 5 m M GZ were used in the mucosal side. 100 m M phosphate buffered solution (pH 7.0) was used in the serosal side. G represents glycerin. Data represent the mean±S.D. of three experiments.

In Vitro Membrane Permeability Study First, in vitro membrane permeability study was performed to clarify different membrane permeability of GZ between the jejunum and colon. Apparent permeability coefficient ($P_{app}$) values in the jejunum and colon were 0.432±0.062×10^{-6}, 0.654±0.056×10^{-6} cm/s (n=6), respectively. Data show that GZ permeability in the colon exceeded that in the jejunum.

Secondly, membrane permeability was examined to clarify which degree the difference in pH of buffered solution affects the $P_{app}$ value of GZ, because GZ solubility increased in dependent on a pH increase in phosphate buffered solution. In addition, the $P_{app}$ value in the colon increased with increased pH (Table 3). The $P_{app}$ value at pH 8.0 was 1.057×10^{-6} cm/s; it was nine times higher than that at pH 6.0. More than predicted, membrane permeability of GZ was promoted remarkably by an increase in pH.

Similarly, the $P_{app}$ of GZ using non-aqueous formulation was examined in rat colon (Table 4). The $P_{app}$ value of GZ from glycerin formulation was remarkably higher (0.379×10^{-6} cm/s) than those of other excipients, but was low as compared to that of GZ formulation prepared with pH 8.0 phosphate buffered solution (Table 3). Furthermore, the $P_{app}$ value of GZ from glycerin-PEG 400 mixture formulation was also low. Although glycerin’s effect on GZ solubility was sufficient, the enhancing effect of glycerin on membrane permeability of GZ was low.

In Vivo Absorption Study The pharmacokinetic profiles
after rectal administration of three GZ formulations dissolved with 400 mM phosphate buffered solution (pH 7.0), glycerin or PEG 400/PG (55 : 5) which is a modified formulation according to the report of Yamamoto et al.\(^{15}\) were examined in rats (Fig. 4A). Because PEG 400/PG (55 : 5) formulation shows useful absorption of GZ, it was chosen as a reference formulation. The plasma concentration of GZ did not show the same profiles after administration of three formulations. The pharmacokinetic parameters are shown in Table 5. Maximum plasma concentrations (C\(_{\text{max}}\)) of GZ after administration of 400 mg/kg phosphate buffer (pH 7.0), glycerin and PEG 400/PG (55 : 5) formulations were 27.9, 20.0 and 14.9 \(\mu\)g/ml, respectively. The times (T\(_{\text{max}}\)) of C\(_{\text{max}}\) of GZ were found to be at 30 min for all formulations. The area under the plasma concentration vs. time curve (AUC) values of GZ in 400 mM phosphate buffer, glycerin and PEG 400/PG (55 : 5) formulations were 94.4±11.6, 94.5±20.3 and 36.3±11.0 \(\mu\)g · h/ml, respectively. According to the result of Yamamura et al.,\(^{12}\) AUC value of GZ after i.v. administration of 50 mg/kg doses was 500.2 \(\mu\)g · h/ml. Comparing the AUC values after i.v. and rectal administrations of 50 mg/kg dose, the bioavailabilities of GZ were calculated to be 18.9±2.32, 18.9±4.05 and 7.25±2.20% for 400 mM phosphate buffer, glycerin and PEG 400/PG (55 : 5) formulation, respectively.

As the results showed different plasma GZ concentration vs. time profiles, absorption rate of GZ from the rectum was evaluated by a deconvolution method using both i.v. and rectal administration data (Fig. 4B). After administration of phosphate buffered formulation, the initial absorption rate of GZ reached to be 1.79±0.42 mg/h. However, thereafter the absorption rate of GZ was rapidly decreased to the similar level as that of glycerin formulation. On the other hand, absorption rate of GZ from PEG 400/PG (55 : 5) formulation was lower than other formulations during experimental period.

### DISCUSSION

The fact that gastrointestinal absorption of GZ is poor after oral administration\(^{10}\) leads to anticipation of enhanced effectiveness of rectal administration, because the effects of gastric juices and various enzymes in the small intestine are avoided by rectal administration. Therefore, preparation of a concentrated GZ solution was tried as one of GZ delivery systems for rectal administration. Fortunately, the \(P_{\text{app}}\) value of GZ in the colon was about 2 folds as compared to that in the jejunum, suggesting that GZ bioavailability would be enhanced in the colon. This result is supported by the report that bioavailability of GZ after intracolonic administration of GZ (50 mg/kg) was significantly higher than that after intraduodenal administration of GZ (50 mg/kg).\(^{16}\)

An important mean to enhance the absorption of GZ in the colon is the increased concentration of GZ in formulation. Increased dosage volume to the colon is impossible from the viewpoint of the capacity. Therefore, it is necessary to increase the GZ concentration in formulation. It has been reported that maximum GZ plasma concentration was approximately 1 \(\mu\)g/ml after oral administration of GZ (50 mg/kg) in rats.\(^{12}\) If rectal administration volume to rats (250 g body weight) is set to e.g. 100 \(\mu\)l, at least 125 mg/ml concentration of GZ will be necessary in a formulation. We set GZ concentration at 200 mg/ml and engaged in preparation of GZ formulation. Results indicated that GZ solubility was increased

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**Table 5. Pharmacokinetic Parameters of Glycyrrhizin (GZ) after Rectal Administration of 50 mg/kg Dose to Rats**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>400 mM phosphate buffer (pH 7.0)</th>
<th>Glycerin</th>
<th>PEG 400/PG (55 : 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> ((\mu)g/ml)</td>
<td>46.8±23.7</td>
<td>42.1±12.9</td>
<td>31.8±9.0</td>
</tr>
<tr>
<td><strong>(\alpha)</strong> (h(^{-1}))</td>
<td>1.14±0.71</td>
<td>1.21±0.24</td>
<td>1.99±0.18</td>
</tr>
<tr>
<td><strong>B</strong> ((\mu)g/ml)</td>
<td>11.3±10.2</td>
<td>11.4±0.33</td>
<td>7.91±1.57</td>
</tr>
<tr>
<td><strong>(\beta)</strong> (h(^{-1}))</td>
<td>0.15±0.05</td>
<td>0.09±0.02</td>
<td>0.19±0.04</td>
</tr>
<tr>
<td><strong>C</strong> ((\mu)g/ml)</td>
<td>58.1±33.9</td>
<td>53.5±13.3</td>
<td>39.8±10.6</td>
</tr>
<tr>
<td><strong>(t_{1/2})</strong> (h)</td>
<td>4.80±1.51</td>
<td>7.88±2.16*</td>
<td>3.86±0.81</td>
</tr>
<tr>
<td><strong>(k_{1})</strong> (h(^{-1}))</td>
<td>4.67±1.79</td>
<td>2.25±0.10</td>
<td>3.71±0.48</td>
</tr>
<tr>
<td><strong>(CL_{\text{total}})</strong> (l/kg · h)</td>
<td>0.54±0.07**</td>
<td>0.54±0.10**</td>
<td>1.46±0.38</td>
</tr>
<tr>
<td><strong>AUC</strong> ((\mu)g · h/ml)</td>
<td>94.4±11.6**</td>
<td>94.5±20.3**</td>
<td>36.3±11.0</td>
</tr>
<tr>
<td><strong>BA</strong> (%)</td>
<td>18.9±2.32**</td>
<td>18.9±4.05**</td>
<td>7.25±2.20</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters were calculated based on the 2-compartment model with first-order absorption process. Data represent the mean±S.D. of three experiments. *\(p<0.05\) and **\(p<0.01\) vs. PEG 400/PG (55 : 5) formulation (1-way ANOVA and Tukey test). BA represents bioavailability, and the plasma GZ concentration after intravenous administration of GZ (50 mg/kg) were used reference values.\(^{12}\)

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**Fig. 5. Photographs of Colon Tissue after in Vivo Absorption Study**

(A) Control (no treatment), (B) at 4 h after rectal administration of GZ from 400 mM phosphate buffer (pH 7.0) formulation (50 mg/0.5 ml/kg GZ).
in 30 to 45% ethanol and was enhanced with increased pH under a weak alkaline condition. By the combination of pH adjustment and ethanol addition, 200 mg/ml GZ formulation was succeeded to prepare in the condition of 100 mM phosphate buffered solution (pH 9.0) containing 30 or 40% ethanol over 30°C. However, the formulation changed to a solution with white turbidity when temperature decreased to 20°C. We inferred that this turbidity change of formulation represents the start of GZ gel-forming. The preparation method with high salt concentration was performed using phosphate buffer, pH 7.0 or pH 8.0, without solubilizer such as surfactants to inhibit the gel-forming of the highly concentrated GZ solution at low temperature such as 10°C. Good correlation was shown between phosphate buffer concentration and GZ gel-forming temperature. These results suggest that the ion strength may inhibit GZ gel-formation. Based on these results, we established a preparation method to obtain high concentrated GZ solution, 200 mg/ml.

A non-aqueous formulation is frequently used in pharmaceutical product. Therefore, preparation of different types of GZ formulation was studied using safe pharmaceutical excipients. GZ was dissolved in glycerin at 200 mg/ml concentration, but not in polyethylene glycol and PG, etc. The viscosity of glycerin formulation is higher than that of phosphate buffered formulation. The increase of luminal viscosity has been reported to inhibit the absorption of warfarin and o-glucose from the intestine.13) Therefore, high viscosity formulation with glycerin may decrease the membrane permeability of GZ. As suggested from the above information, the P app value of GZ from glycerin formulation was low (0.379×10⁻⁶ cm/s). Absorption improvement of GZ in the colon cannot be expected.

In vivo absorption of GZ from 400 mM phosphate buffer (pH 7.0) and glycerin formulations was examined after application to rat rectum. PEG 400/PG (55 : 5) formulation was chosen as a reference preparation. Although the same dose of GZ (50 mg/kg) was administered to rats, C max decreased in the order of 400 mM phosphate buffer (pH 7.0), glycerin and PEG 400/PG (55 : 5) formulations. Difference in the C max was similar to that of P app values obtained from in vitro experiments, suggesting that a higher membrane permeability of GZ from rectum provided a higher C max. The results that the k values obtained after the administration of 400 mM phosphate buffer (pH 7.0) formulation was higher than those of other formulations also show high absorption rate of GZ. The absorption rate of GZ is thought to be affected by the membrane permeability of GZ. In order to clarify the difference in the absorption rate of GZ from rectum, deconvolution analysis was performed. As the results, the differences in the absorption rate of GZ observed in the three formulations can be attributed to the membrane permeability of GZ obtained in the in vitro study. Generally, when absorption rate of drug is decreased by changing the drug formulation, prolonged T max is obtained. However, there was no difference on T max among three formulations. To explain this result, the contribution of β-glucuronidase to the metabolism of GZ in first-pass effect was considered. It is known that GZ is metabolized to glycyrrhetic acid in several organs such as liver, intestine, spleen, etc.18) For example, the plasma concentration of glycyrrhetic acid increased rapidly after oral administration of GZ to rats.19) Therefore, it seems that plasma GZ concentration is dependent on metabolism by the enzymes. The t 1/2 of GZ after administration of glycerin formulation was longer than those obtained after the administration of other formulations. The AUC of GZ after administration of PEG 400/PG (55 : 5) formulation was smaller than those obtained after the administration of other formulations. From both results, it is suggested that t 1/2 was prolonged by sustained absorption of GZ and almost all of GZ was absorbed from the rectum in glycerin formulation. In contrast, GZ absorption from the rectum was incomplete and sustained absorption was not obtained in PEG 400/PG (55 : 5) formulation. The result that low GZ bioavailability after administration of PEG 400/PG (55 : 5) formulation supports the possibility of incomplete absorption of GZ. In fact, small amount of PEG 400/PG (55 : 5) formulation was remained visually near the anus after in vivo experiments. On the other hand, we inferred that the phosphate buffered formulation enhanced C max and bioavailability of GZ in rat colon.

Fujioka et al. reported that maximum plasma GZ concentrations after rectal administration (80, 500 mg) to human volunteers were 2 and 5 μg/ml, respectively, and they showed the usefulness of anti-inflammatory agents for chronic hepatitis by rectal administration of GZ.20) From the results of plasma GZ concentration after administration of a phosphate buffered formulation, GZ concentration over 2 μg/ml was maintained up to 4 h. This plasma concentration, maybe, provides enough anti-inflammatory effect. Moreover, histological examination was carried out to examine the safety of GZ formulation. The phosphate buffered formulation did not cause any failure of the mucosa of the large intestine.

In conclusion, GZ formulation having, high concentration of GZ 200 mg/ml succeeded using highly concentrated (≥350 mM) phosphate buffered solution, pH 7.0, or glycerin. Membrane permeability of GZ using the phosphate buffered formulation was higher than that of the glycerin formulation. Furthermore, phosphate buffered formulation enhanced the bioavailability of GZ without morphological damage to the large intestine. Therefore, a highly concentrated GZ solution using phosphate buffered formulation may be useful as a new therapeutic system.

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