DOES BACITRACIN HAVE AN ABSORPTION-ENHANCING EFFECT IN THE INTESTINE?
Shinji GOTOH, Reiko NAKAMURA, Mizue NISHIYAMA, Takuya FUJITA, Akira YAMAMOTO* and Shozo MURANISHI
Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

We studied the absorption enhancement effects of three types of protease inhibitors, aprotinin, bacitracin and soybean trypsin inhibitor, in the rat intestine. Of these protease inhibitors, bacitracin enhanced the absorption of FD-4 and phenol red from the rat small and large intestine without mucosal toxicity. Thus, it was suggested that bacitracin has not only a protease-inhibitory but also an absorption-enhancing capability.

**KEY WORDS** protease inhibitor; bacitracin; fluorescein isothiocyanate dextran; phenol red; intestinal absorption; absorption enhancement

The oral bioavailability of peptide and protein drugs is generally poor. This has been attributed to their extensive proteolytic degradation in the gastrointestinal tract and their low permeability across the intestinal mucosa.1, 2) To overcome these delivery problems, various approaches have been examined. Of these approaches, protease inhibitors have been used for improving the absorption of peptide and protein drugs from the gastrointestinal tract.3-5) This effect was mainly explained by the enhanced stability of peptides due to the reduced activities of proteases. Alternatively, it may be possible that, like Na-glycocholate, these protease inhibitors also have absorption-enhancing activities besides their proteolytic activities.6) However, few studies have been examined on the absorption-enhancing actions of protease inhibitors.

In this study, phenol red (M.W.; 354: PR ) and fluorescein isothiocyanate dextran with average molecular weight of 4,000 (FD-4) were chosen as poorly absorbable and stable model drugs, and the effects of protease inhibitors on the absorption of these drugs from the small and large intestine were examined in rats. We also studied the intestinal mucosal damage caused by these protease inhibitors.

**MATERIALS AND METHODS**

**Preparation of Drug Solution** FD-4 and PR were dissolved in isotonic phosphate buffer (small intestine: pH 6.5, large intestine: pH 7.4) to yield the final concentration of 2 mg/rat. In certain experiments, the dosing solutions were added with protease inhibitors such as aprotinin (0.5 mg/ml), bacitracin (10 mM, 20 mM), or soybean trypsin inhibitor (STI: 10 mg/ml) according to our previous report.3)

**Absorption Experiments** Absorption experiments were performed by an *in situ* closed loop method using male Wistar rats (200-250 g).7) A small or large intestinal loop was prepared by cannulation with silicone tubing at the proximal and distal ends of the small and large intestine, respectively. Five or 2 ml of the drug solution at 37 °C were injected into small or large intestinal loops, respectively. A cannulation was also made in the jugular artery with a polyethylene tube. After administration, blood was periodically taken from the jugular artery. The plasma was separated by centrifugation, and the drug concentration in the plasma was determined.

**In Vivo Intestinal Mucosal Toxicity Experiments** The intestinal mucosal toxicity of protease inhibitors was evaluated by the leakage of Evans blue (EB) from the systemic circulation.8, 9) Male Wistar rats, weighing 250-300 g, were used in this study. After 0.5 ml of 3 % EB was injected intravenously into rats, 5 or 2 ml of the buffer solution at 37°C was administered into small or large intestinal loops, respectively. Four hours after the administration, the solution in the intestinal loop was washed with isotonic phosphate buffer and the concentrations of EB in this solution were determined spectrophotometrically.

*To whom correspondence should be addressed. © 1995 Pharmaceutical Society of Japan
Analytical Methods  The fluorescence intensity of FD-4 was measured on a fluorescence spectrophotometer at excitation wavelength of 495 nm and emission wavelength of 512 nm, respectively. PR of a plasma sample solution was alkalinized with 3 ml of 1 N NaOH and determined spectrophotometrically at 560 nm. EB in phosphate buffer was extracted with acetone, and these samples were centrifuged and determined spectrophotometrically at 606 nm.

RESULTS AND DISCUSSION
Effects of Protease Inhibitors on the Small and Large Intestinal Absorption of PR  Figure 1 shows the effects of protease inhibitors on the concentration-time profiles of PR following small intestinal administration in rats. As shown in Fig. 1, the absorption of PR from the small intestine was enhanced by bacitracin; however, neither aprotinin nor STI showed any marked effect on the absorption of PR. Similar results were observed on its absorption from the large intestine. Tables 1 and 2 summarize various pharmacokinetic parameters following the small or large intestinal administration of PR with or without protease inhibitors. In the small intestine, a significant increase in AUC value was noted in the presence of bacitracin (10 and 20 mM) (Table 1), and similar results are also obtained in the large intestine (Table 2). These results suggest that bacitracin shows not only a protease inhibitory activity but also an absorption enhancing activity.

Effects of Protease Inhibitors on the Small and Large Intestinal Absorption of FD-4  The absorption of FD-4 from the small and large intestine was also enhanced by bacitracin in the same manner as was the absorption of PR. In the small and large intestine, the AUC values in the presence of 10 mM bacitracin were 3.4 and 2.6 times greater than those without bacitracin, and the degree of absorption-enhancing effect of bacitracin for FD-4 was higher than that for PR. These results might indicate that bacitracin enhanced absorption of macromolecular drugs such as FD-4 rather than low molecular weight drugs such as PR.

![Graph](image-url)

Fig. 1. Plasma Concentration-Time Profiles of PR after Small Intestinal Administration in the Presence of Various Protease Inhibitors. Results are expressed as the mean±S.D. (n=4). Key: ●, Control; □, Bacitracin (10 mM); ■, Bacitracin (20 mM); ○, Aprotinin (0.5 mg/ml); △, STI (10 mg/ml)
Table 1. Pharmacokinetic Parameters after Small Intestinal Administration of PR

<table>
<thead>
<tr>
<th></th>
<th>C_{max} (µg/ml)</th>
<th>T_{max} (min)</th>
<th>AUC_{0-240} (µg/ml*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.02 ± 0.15</td>
<td>195 ± 45.0</td>
<td>345 ± 21.5</td>
</tr>
<tr>
<td>Bacitracin (10 mM)</td>
<td>3.05 ± 0.29</td>
<td>52.5 ± 7.50</td>
<td>494 ± 63.2*</td>
</tr>
<tr>
<td>Bacitracin (20 mM)</td>
<td>4.46 ± 0.12</td>
<td>52.5 ± 14.4</td>
<td>667 ± 5.50***</td>
</tr>
<tr>
<td>STI (10 mg/ml)</td>
<td>1.47 ± 0.11</td>
<td>187 ± 33.3</td>
<td>250 ± 22.0</td>
</tr>
<tr>
<td>Aprotinin (0.5 mg/ml)</td>
<td>1.70 ± 0.09</td>
<td>135 ± 60.6</td>
<td>321 ± 25.2</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. of 4 experiments.  
*P<0.05,  ***P<0.001, significantly different from the control.

Table 2. Pharmacokinetic Parameters after Large Intestinal Administration of PR

<table>
<thead>
<tr>
<th></th>
<th>C_{max} (µg/ml)</th>
<th>T_{max} (min)</th>
<th>AUC_{0-240} (µg/ml*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.97 ± 0.08</td>
<td>191 ± 34.3</td>
<td>136 ± 11.6</td>
</tr>
<tr>
<td>Bacitracin (10 mM)</td>
<td>1.26 ± 0.06</td>
<td>37.5 ± 7.50</td>
<td>175 ± 12.3*</td>
</tr>
<tr>
<td>Bacitracin (20 mM)</td>
<td>3.81 ± 0.59</td>
<td>30.0 ± 0.03</td>
<td>445 ± 35.3***</td>
</tr>
<tr>
<td>STI (10 mg/ml)</td>
<td>0.58 ± 0.06</td>
<td>203 ± 9.70</td>
<td>94.1 ± 11.5</td>
</tr>
<tr>
<td>Aprotinin (0.5 mg/ml)</td>
<td>1.20 ± 0.14</td>
<td>143 ± 49.6</td>
<td>174 ± 34.0*</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.D. of 4 experiments.  
*P<0.05,  ***P<0.001, significantly different from the control.

On the other hand, the enhancement effect of bacitracin on the intestinal absorption of FD-4 was more clearly observed in the small intestine than in the large intestine.

In Vivo Intestinal Mucosal Toxicity Experiments  The effects of various protease inhibitors on intestinal mucosal toxicity were examined by measuring the leakage of EB from the systemic circulation.  In the small and large intestine, the leakage amounts of EB in the presence of bacitracin were one-third and one-fifty-second-fold, respectively, less than that in the case of BL-9 as a positive control.  No significant difference in the leakage of EB was observed between bacitracin and other protease inhibitors.  This finding suggested that mucosal damage caused by these protease inhibitors was less than that of BL-9 in the small and large intestine.

In conclusion, the present study demonstrated that improvement of intestinal absorption of peptides by bacitracin may be explained by its absorption-enhancing action in addition to its protease inhibitory action.  Furthermore, bacitracin may be a suitable adjuvant for improving the intestinal absorption of poorly absorbable drugs including peptides, as it did not cause serious intestinal mucosal damage.

REFERENCES


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