Regular Article

Inhibitory Effect of Zinc on the Absorption of β-Lactam Antibiotic Ceftibuten via the Peptide Transporters in Rats

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Summary: Zinc is an essential metal ion for the body, and is widely used for nutritional and clinical purposes. Previously, we showed that zinc inhibits the transport of glycylsarcosine via the intestinal peptide transporter PEPT1 in the human intestinal cell line Caco-2. In this study, we examined the effect of zinc on the activity of peptide transporters in rats using the oral β-lactam antibiotic ceftibuten as a model drug. The plasma ceftibuten concentration after intraintestinal administration was decreased in the presence of zinc. The maximum plasma concentration (Cmax) was significantly decreased and the time required to reach Cmax (Tmax) was prolonged by zinc coadministration. The plasma ceftibuten concentration after iron coadministration or two hours after zinc administration was not affected. The in situ loop technique revealed 50% inhibition of ceftibuten absorption by zinc. In conclusion, zinc inhibits the transport activity of PEPT1 in vivo as well in vitro.

Keywords: zinc inhibition; peptide transporters; β-lactam antibiotics; small intestine; drug interaction

Introduction

Zinc is an essential metal ion necessary for growth, development, immune function, and for many biological cellular functions. Zinc deficiency in humans results in retarded growth, male hypogonadism, skin changes, poor appetite, mental lethargy, and lack of taste and smell.1) Since these symptoms are recovered when zinc is administered, zinc is clinically used for its deficiency. Numerous clinical studies found that zinc supplementation for infants, young children, and pregnant and lactating women results in various positive and clinically relevant effects.2,3) On the other hand, zinc interacts with many kinds of proteins such as intestinal transporters. For example, inhibitory effects of zinc on the intestinal transport of glucose,4) L-threonine,5) and folic acid6) have been reported.

Previously, we revealed that zinc exerts an inhibitory effect on the intestinal peptide transporter PEPT1 using the human intestinal cell line Caco-2.7) It has been demonstrated that PEPT1 greatly contributes to the protein homeostasis8) and the therapeutic efficacy of peptide-like drugs.9) As peptide-like drugs, β-lactam antibiotics, the anticancer agent bestatin, and the antivirus agent valacyclovir were reported to be transported by PEPT1.10–12) Interactions between zinc and peptide transporters may result in negative effect on the nutritional and clinical aspects of small intestinal function. However, little is known about the interactions between zinc and small peptides or peptide-like drugs in vivo.

In the present study, we examined the effect of zinc on the absorption of the β-lactam antibiotic ceftibuten in rats. β-lactam antibiotics are often used as useful probes to characterise the role of peptide transporters because these antibiotics are resistant to hydrolysis in the gut.9) The present data showed that zinc decreases the transport activity of peptide transporters in vivo, and this interaction should be considered when zinc is used as a supplement for the treatment of malnutrition.

Materials and Methods

Materials: Ceftibuten was a gift from Shionogi (Osaka, Japan). ZnSO4 and FeSO4 were purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity available.

Animals: Male Wistar rats weighing 200 to 230 g were used. Rats were fasted overnight before ceftibuten administration but given free access to water. Blood sam-
samples were collected from the femoral artery. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University, and the experimental protocol was approved by the Animal Research Committee of the Graduate School of Medicine of Kyoto University (Med Kyo 04339).

**Pharmacokinetic studies in rats:** Rats fasted overnight for at least 15 h were anesthetized with 50 mg/kg sodium pentobarbital. Their body temperature was maintained with appropriate heating lamps. The femoral artery was cannulated with a polyethylene tube (SP-31, Natsume Seiskusho, Tokyo, Japan) for blood sampling. The abdominal cavity was opened via a middle incision, and the upper duodenum was ligated twice with silk sutures (4–0 Nescosuture®, Nihon-shoji, Osaka, Japan). Then, drug solution was administered at 3 ml/kg into the middle part of the duodenum. The composition of the buffer was as follows (mM): 145 NaCl, 3 KCl, 1 CaCl₂, 0.5 MgCl₂, 5 D-glucose, and 5 N-2-hydroxyethylpiperazine-N’2-ethanesulfonic acid (HEPES; adjusted to pH 6.5). Rats were given ZnSO₄ (4.5 mg/kg) or buffer at first, and thereafter ceftibuten (1.5 mg/kg) was administered. Blood samples were collected from the femoral artery 15, 30, 45, 60, 90, 120, 180, and 240 min after the administration of ceftibuten. Samples of 200–300 µl in volume were collected in heparin tubes and then centrifuged for 3 min at 14,000 × g. The plasma obtained was used for the determination by high-performance liquid chromatography.

**In situ loop technique:** Intestinal absorption of ceftibuten was assessed by the in situ loop technique as described previously. A cannula with a polyethylene tube was inserted in the portal vein. A duodenum loop 20 cm in length was prepared, and then ZnSO₄ (4.5 mg/kg) and ceftibuten (1.5 mg/kg) were introduced into the loop with a microsyringe. Blood was withdrawn from the portal vein at designated times in heparin tubes and was centrifuged for 3 min at 14,000 × g. The plasma obtained was used for the determination by high-performance liquid chromatography.

**Analytical methods:** Plasma (100 µl) was mixed with methanol (200 µl) and centrifuged for 3 min at 14,000 × g. The supernatant (50 µl) was analyzed by high-performance liquid chromatography (LC-10A, Shimadzu Co., Kyoto, Japan) with Zorbax ODS columns, 15 cm × 4.6 mm (Du Pont, Wilmington, DE) as previously described.

**Pharmacokinetic analysis:** The pharmacokinetic parameters of ceftibuten were calculated by non-compartment analysis based on statistical moment theory. The area under the plasma concentration-time curve (AUC) after intraintestinal administration was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the ceftibuten concentration at the last sampling time (C₂₄₀) to the terminal elimination rate constant (kₑ). The mean residence time (MRT) was determined using the relationship of AUMC/AUC, where AUMC is the area under the first moment curve (plasma concentration × time vs. time). The highest observable concentration and associated time point were defined as the maximum concentration (Cᵢₘₐₓ) and corresponding time (Tᵢₘₐₓ), respectively.

**Data analysis:** Each experimental point shown in the figures represents the mean ± SE of four to six experiments. When the error bars were smaller than the symbols, they were not shown. The data was statistically analyzed by the non-paired t test.

**Results**

**Figure 1** shows the time course of the ceftibuten plasma concentration after intraintestinal administration in the absence or presence of zinc. We introduced 4.5 mg/kg of ZnSO₄ (1.0 mg/kg of zinc) to elucidate the effect of zinc dosage for the treatment of malnutrition. The initial absorption rates were significantly decreased by zinc. The pharmacokinetic parameters of ceftibuten after intraintestinal administration are summarized in **Table 1**. The Cᵢₘₐₓ and the AUC from 0 to 60 minutes were significantly decreased in the presence of zinc, but the AUC estimated to infinity was not significantly changed. The Tᵢₘₐₓ, T½, and MRT tended to be prolonged but not significantly.

We also examined the effect of iron on the ceftibuten plasma concentration after intraintestinal administration. In contrast to zinc coadministration, as shown in **Figure 2**, the ceftibuten plasma concentration was not inhibited by iron and the pharmacokinetic parameters were not changed either (**Table 1**).

Next, we examined the timing of zinc administration
Table 1. Pharmacokinetic parameters of ceftibuten after intraintestinal administration with zinc or iron

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ZnSO₄</th>
<th>FeSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (min)</td>
<td>37.5 ± 3.4</td>
<td>60.0 ± 12.2</td>
<td>48.8 ± 7.2</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>1.24 ± 0.07</td>
<td>0.974 ± 0.080*</td>
<td>1.39 ± 0.05</td>
</tr>
<tr>
<td>T½ (min)</td>
<td>48.5 ± 5.0</td>
<td>71.7 ± 19.1</td>
<td>63.0 ± 16.4</td>
</tr>
<tr>
<td>AUC₀–₆₀ (µg min/ml)</td>
<td>57.9 ± 3.3</td>
<td>39.7 ± 2.9**</td>
<td>55.9 ± 4.8</td>
</tr>
<tr>
<td>AUCinf (µg min/ml)</td>
<td>148 ± 8</td>
<td>166 ± 38</td>
<td>190 ± 43</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>91.8 ± 6.0</td>
<td>135 ± 24</td>
<td>116 ± 28</td>
</tr>
</tbody>
</table>

Ceftibuten (1.5 mg/kg) was administrated with or without ZnSO₄ or FeSO₄ (4.5 mg/kg). Each value represents the mean ± SE of four to six rats.

*p < 0.05,  **p < 0.005, significantly different from the control.

Fig. 2. The effect of iron coadministration on the ceftibuten plasma concentration after intraintestinal administration
At first, FeSO₄ (4.5 mg/kg) (●) or buffer (○) was administrated, and ceftibuten (1.5 mg/kg) was administrated immediately after. Thereafter, blood samples were collected at 15, 30, 45, 60, 90, 120, 180, and 240 min and the ceftibuten concentrations were determined. Each point represents the mean ± SE of four to five rats.

Fig. 3. The effect of zinc pretreatment on the ceftibuten plasma concentration after intraintestinal administration
At first, ZnSO₄ (4.5 mg/kg) (●) or buffer (○) was administrated, and ceftibuten (1.5 mg/kg) was administrated 2 hours later. Thereafter, blood samples were collected at 15, 30, 45, 60, 90, 120, 180, and 240 min and the ceftibuten concentrations were determined. Each point represents the mean ± SE of four rats.

Fig. 4. The effect of zinc coadministration on the ceftibuten concentration in the portal vein by in situ intestinal loops after intraintestinal administration
At first, ZnSO₄ (4.5 mg/kg) (●) or buffer (○) was administrated, and ceftibuten (1.5 mg/kg) was administrated immediately after. Thereafter, blood samples were collected from the portal vein at 1, 3, 6, 9, 12, 15, and 30 min and the ceftibuten concentrations were determined. Each point represents the mean ± SE of four rats.

on the ceftibuten plasma concentration. As shown in Figure 3, the ceftibuten plasma concentration was not changed when zinc was administered two hours before ceftibuten administration. Pharmacokinetic parameters were also not changed compared with the control (control vs ZnSO₄: Cmax (µg/ml), 1.09 ± 0.03 vs 1.07 ± 0.07; T½ (min), 68.2 ± 4.8 vs 70.6 ± 8.4; AUCinf (µg min/ml), 159 ± 7 vs 170 ± 15).

Finally, the effect of zinc on the intestinal absorption of ceftibuten was assessed by in situ intestinal loops to focus on the intestinal absorption process. Figure 4 shows the mean portal vein concentrations after intraduodenal administration of ceftibuten. The initial absorption rate of ceftibuten was significantly reduced by zinc coadministration, suggesting that the changes in the bioavailability parameters were related to the decrease of intestinal absorption of ceftibuten.

Discussion

Previously, we showed that zinc is a competitive inhibitor of the intestinal peptide transporter PEPT1 and exerts a moderate inhibitory effect on the intestinal basolateral peptide transporter using the human intestinal epithelial cell line Caco-2.7) These observations suggest that the absorption of dipeptides or peptide-like drugs may be reduced when zinc is coadministered. In the present study, we clearly demonstrated that zinc decreased the initial absorption rate and maximum plasma concentration of ceftibuten after intraintestinal administration. This inhibition disappeared when zinc was administered two hours before ceftibuten administration, suggesting that the inhibitory effect of zinc on the peptide transporters was reversible and that zinc was removed or diluted at the ceftibuten absorption site of the intestine within two hours. These findings strongly sug-
gested that zinc competitively inhibited PEPT1 in vivo as shown in the cell line system.

Divalent and trivalent pharmaceutical cations such as Zn$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ interact with new quinolone antibacterials$^{(4)}$ or tetracycline antibiotics$^{(5)}$ by chelation, and reduce the intestinal absorption of these drugs. There is a possibility that the interaction between zinc and ceftibuten is due to the decreased solubility of ceftibuten caused by its chelation with zinc. However, as previously reported, inhibitory mechanism of zinc on PEPT1 was due to a direct interaction with this transporter.$^{7}$ We speculated that zinc competitively inhibited the H$^+$ binding to the histidine residue of PEPT1 proteins. Therefore, it is likely that zinc affects drug absorption not only by chelating drugs but also by modulating the function of PEPT1.

When zinc and ceftibuten were coadministered, the $C_{max}$ of ceftibuten was decreased to 80%. In the case of $\beta$-lactam antibiotics, it is important to maintain sufficient plasma concentrations of these drugs to prevent the appearance of resistant bacteria. Therefore, to avoid ceftibuten and zinc interaction, the timing of ceftibuten and zinc administration should be considered.

Numerous studies have found that the absorption of protein digestion products occurs primarily in the form of small di- and tripeptides$^{(8,16,17)}$ and that the rate of absorption of amino acid is greater for the dipeptide than for the corresponding mixture of free amino acids,$^{(18)}$ indicating that dipeptides need not be hydrolysed before absorption. Therefore, the intestinal peptide transporter PEPT1 plays a major role in protein digestion by transferring dipeptides from the lumen to intestinal epithelial cells. We also found that another peptide transporter was expressed in the basolateral membranes of intestinal epithelial cells and that PEPT1 and this transporter cooperate in the efficient transepithelial transport of small peptides and various peptide-like drugs.$^{19,20}$ In this study, we observed an inhibitory effect of zinc on PEPT1 in vivo, suggesting that zinc may interfere with protein absorption.

Zinc supplements are becoming more commonly used, and large pharmacological doses can be taken as mineral supplements.$^{1-3}$ In developing countries, zinc deficiency may be common and associated with immune impairment and increased risk of serious infectious diseases for children, and children received high concentrations of zinc such as 4–5 mg zinc acetate/kg/day or 3 mg zinc sulfate/kg/day.$^{21}$ During pregnancy, zinc supplementation is administered at 30 mg elemental zinc per day.$^{22}$ When the high dose of zinc is administrated for therapeutic purposes, zinc concentration in the gut lumen is supposed to be reached in the mM order. We reported that IC$^{50}$ values of zinc for PEPT1 were 3.4 mM in Caco-2 cells or 1.7 mM in rat PEPT1-expressing oocytes.$^{23}$ Therefore, zinc-drug interaction should be taken into consideration when high dose of zinc is used.

In conclusion, the present data show that the interaction between zinc and peptide transporters occurs in vivo as in vitro model. Clinical assessment for these interactions should be needed since no information is yet available. In addition, investigation of the effects of long-term administration of zinc on the function of peptide transporters would provide useful information for the nutritional and clinical use of zinc.

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References


