Decrease in Ciprofloxacin Absorption by Polyvalent Metal Cations Is Not Fully Attributable to Chelation or Adsorption

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Summary: The drug interaction between new quinolone antibiotics (NQs) and polyvalent metal cation products, leading to a significant decrease in the absorption of NQ, is considered to be attributable to the formation of poorly absorbable chelate and physicochemical adsorption of NQs to cation products. To clarify the mechanisms of this drug interaction in detail, we investigated the effects of Al³⁺ or Mg²⁺ on the membrane permeation profile of ciprofloxacin (CPFX) across human colon carcinoma cell lines (Caco-2) in monolayer culture, and characterized the adsorption nature of CPFX to polyvalent metal cation products under physiological conditions. As a result, Al³⁺ or Mg²⁺ partially but not fully impaired the permeation of CPFX across Caco-2 monolayer up to 30% or 60% of control, respectively. Physicochemical adsorption of CPFX to cation products was not observed under physiological pH. In conclusion, two possible mechanisms investigated, the decrease in the permeability of CPFX by chelate formation and adsorption of CPFX to polyvalent metal cation products, may partially but not fully explain the extent of the drug interaction clinically observed.

Keywords: drug interactions; absorption; Caco-2 cells; permeability; adsorption; new quinolones; aluminum; magnesium; chelate

Introduction

New quinolone antibiotics (NQs) are frequently used for the treatment of a variety of bacterial infections because of their broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria.1 On the other hand, previous studies have demonstrated that the area under the plasma concentration curve (AUC) of NQs, the maximum plasma concentration (Cmax) and bioavailability are decreased by the coadministration of antacids when they are concomitantly administered under the standard dosage range. When concomitantly administered within their clinical dosage ranges, almost all NQ molecules conceivably form chelates with metal cations in the GI tract. With regard to physicochemical adsorption, Toyoguchi et al. reported in an in vitro study in 0.05 M phosphate buffer at pH 6.8 that 7%, 33% or 88% of CPFX was adsorbed to aluminum hydroxide gel, sucralfate or aluminum silicate, respectively. However, phosphate in the buffer may have affected the adsorption nature by forming an insoluble complex with CPFX. Therefore, their results may not simply reflect the adsorption.

The decrease in the in vitro permeability of NQs across the intestinal epithelial cell monolayer by forming chelates with certain polyvalent metal cations remains to be investigated quantitatively. In addition no researchers have quantitatively evaluated physicochemical adsorption of NQs to polyvalent metal cation products under clinically feasible conditions.

The aim of this study was to evaluate quantitatively the contribution of chelate formation and physicochemical adsorption on DDI between NQs and polyvalent metal cations. First we evaluated quantitatively the effects of Al³⁺ or Mg²⁺ on the membrane permeation profiles of a typical NQ, CPFX, across human colon carcinoma cell lines (Caco-2) in monolayer culture. Second, we assessed the amount of CPFX adsorbed to polyvalent metal cation products (Alumigel® or Maglax®) under conditions considered to

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be physiological in the GI tract, based on the assumption that chelation occurs between the solute form of CPFX and polyvalent metal ions while physicochemical adsorption of CPFX occurs with the solid form of polyvalent cation products.

Materials and Methods

Materials: Ciprofloxacin hydrochloride (CPFX) monohydrate and caffeine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Aluminum sulfate (dehydrate) and magnesium oxide were purchased from Nacalai Tesque Ltd. (Tokyo, Japan). Alumigel® Fine Granule 99% (dried aluminum hydroxide gel) was purchased from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). Maglax® Fine Granule 99% (dried aluminum hydroxide gel) was purchased from Yoshida Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals and reagents used were special or high-grade purity from commercial sources.

Cell cultures: Caco-2 cells were purchased from RIKEN Biore资源 Center cell bank (Ibaraki, Japan). The cells were cultured in Dulbecco’s Modified Eagle’s medium (Nacalai Tesque) supplemented with 10% fetal calf serum (Hana-nesco Bio Ltd., Tokyo, Japan), 1% non-essential amino acids (Wako) and 1% penicillin-streptomycin solution (Nacalai Tesque), at 37°C under humidified atmosphere with 5% CO₂. Caco-2 cells between passages 49 through 57 were used for all permeability studies.

To prepare the cell monolayers, Caco-2 cells were seeded at a density of 6.3 × 10⁴ cells/cm² onto Transwell® (cell culture insert surface 4.2 cm², 3 μm pore size) (Corning, Tokyo, Japan). The cells were incubated for 14 to 21 days after initial seeding at 37°C under humidified atmosphere with 5% CO₂. To ensure the epithelial integrity of Caco-2 monolayers, transepithelial electrical resistance (TEER) value was monitored using an ohmmeter (Millicell-Easy Reader, Millipore, Billerica, MA) during incubation. TEER was calculated from the following equation:

\[ \text{TEER} = \left( R_{\text{mono}} - R_{\text{blank}} \right) \times A \]  

where \( R_{\text{mono}} \) is the resistance in the presence of both the cell monolayer and filter, \( R_{\text{blank}} \) is the resistance of the filter itself, and \( A \) is the surface area of the filter (4.67 cm²).

Monolayers having TEER values less than 800 Ω·cm² were not used in the study.

Permeability profiles: The permeability experiments were carried out under the following protocol as previously described. Briefly, after stabilization in an aliquot of 2 mL of low-Mg/Ca HEPES-Tris buffer (140 mM NaCl, 5 mM KCl, 0.25 mM CaCl₂, 0.1 mM MgCl₂, 10 mM d-glucose, 10 mM HEPES, 10 mM Tris, pH 6.8) in apical and basal chambers at 37°C for 15 min, permeability assays were initiated by replacing the apical solution with pH 6.8) in apical and basal chambers at 37°C for 15 min, permeability assays were initiated by replacing the apical solution with

Moreover, FITC-inulin was spiked to the apical side (final concentration: 0.25 mg/mL) and used as a paracellular marker to ensure that the monolayers remained intact until 120 min. TEER was measured at the end of each experiment.

Analysis of FITC-inulin by fluorometry: The concentration of FITC-inulin was quantitatively determined by a spectrophotometer (infinite M1000; Tecan Japan Co., Ltd., Kanagawa, Japan) with excitation at 485 nm and emission at 530 nm. The quantitation limit was 0.25 μg/mL.

Analysis of CPFX by HPLC-UV method: The HPLC system consisted of a pump (LC-10A vp, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A vp spectrophotometer, Shimadzu), an octadecylsilane column (Cosmosil, 5C18-AR-II, 4.6 × 150 mm; Nacalai Tesque, Tokyo, Japan) and a column oven (CTO-10A vp; Shimadzu) set at 40°C. The mobile phase consisted of 0.1 M (NH₄)₂HPO₄ (adjusted to pH 3.0 using phosphoric acid), acetonitrile and methanol (83:10:7, v/v), as previously reported, and was pumped at a rate of 1.2 mL/min. The absorbance of CPFX was measured at 278 nm. Concentrations were determined using the peak area ratio of the analyte (CPFX) to an internal standard (5 µM caffeine). The quantitation limit was 0.03 µM. As well, preliminary experiments confirmed that polyvalent metal ions did not affect the UV absorption of CPFX at a wavelength of 278 nm.

Calculation of apparent permeability coefficient (P_app): Permeation rates were calculated from the slope of the plot of the amount CPFX permeated versus time between 0 min and 60 min, where permeation becomes the steady-state. P_app was calculated from the following equation:

\[ P_{\text{app}} (\text{cm/s}) = \frac{dQ}{dt} \times \frac{1}{A \cdot C_0} \]  

where \( \frac{dQ}{dt} \), \( C_0 \) and \( A \) represent the permeation rate (µmol/s), initial CPFX concentration on the apical chamber (µmol/cm²), and the surface area of the monolayer (cm²), respectively.

Physicochemical adsorption profiles: First, 100 mL of Solution I (artificial gastric fluid; 2.0 mg of NaCl and 7 mL of 35% HCl in 1 L of distilled water, pH 1.2) or Solution II (artificial enteric fluid; 10.9 mM citric acid, pH adjusted to 6.8 by NaOH) was spiked with CPFX to give a final concentration of 2.0 mM and stirred for 2 min at 37°C using a magnetic stirrer with a heater (RCH-3, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Secondly, Alumigel® or Maglax® was added to achieve a final concentration of 0.3, 1, 3, 10 or 30 mM (as Al³⁺ or Mg²⁺) and further stirred for 20 min at 37°C under the monitoring of pH and appearance. Finally, 1.6 mL of the mixture was collected and centrifuged at 1,800 × g for 10 min at 32°C. After filtration of the supernatant using a membrane filter (Cosmonice Filter W, 0.45 µm; Nacalai Tesque), the sample was diluted 300-fold (Solution I) or 50-fold (Solution II) and submitted to the determination of CPFX concentration by using a UV-visible spectrophotometer (UV-1800; Shimadzu) at a wavelength of 278 or 272 nm for Solution I or Solution II, respectively. The quantitation limit was 0.3 µM.

In addition, the concentration of each drug was determined from the standard dose (CPFX: 750 mg, Alumigel®: 1 g, Maglax®: 0.3 g) and the volume of the GI tract, which was assumed to be 1 L.

Results

Permeability profiles: Figure 1 shows the effects of aluminum or magnesium concentration on the P_app of CPFX. While Al³⁺ suppressed the P_app of CPFX to 28.9% of the control, the inhibitory dampening effect was not observed with Mg²⁺.
effect of Al$^{3+}$ reached almost the maximum at 0.5 mM. On the other hand, the Papp remained at 60.2% of the control at the highest Mg$^{2+}$ concentration tested.

In all experiments, the Papp values of FITC-inulin were less than 0.02% of the Papp for CPFX alone. Neither Al$^{3+}$ nor Mg$^{2+}$ affected the TEER value measured at the end of the experiments.

Physicochemical adsorption profiles: Figure 2 shows the concentration profiles of CPFX in the supernatant taken from Solution I (pH 1.2) spiked with 2.0 mM CPFX and polyvalent metal cation product [Alumigel (Fig. 2a) or Maglax (Fig. 2b)] at various concentrations. Figures 2c and 2d show the results taken from Solution II (pH 6.8). In Solution I, CPFX concentration in the supernatant was approximately 2.0 mM, which was consistent with the spiked concentration in any amount of Alumigel (Fig. 2a) or Maglax (Fig. 2b), suggesting that CPFX was completely dissolved. It should be also noted that the pH of Solution I was not affected by the addition of polyvalent metal cations.

On the other hand, in Solution II, CPFX concentration in its supernatant was only 0.435 mM, showing that only 22% of the spiked CPFX was dissolved into Solution II (Figs. 2c and 2d). In addition, the effects of Alumigel and Maglax were distinctive; Alumigel increased the CPFX concentration in the supernatant up to 1.68 mM (84% of that added), in a dose-dependent manner (Fig. 2c). In contrast, Maglax decreased the CPFX concentration in the supernatant in a dose-dependent manner only up to 0.028 mM (1% of that added) (Fig. 2d).

In neither Solution I nor Solution II, did Alumigel and Maglax completely dissolve.

With regard to pH, Alumigel did not affect the pH of Solution II containing 2 mM CPFX, while 30 mM Maglax increased the pH of the solution up to 11.1.
Discussion

In this study, we first quantitatively evaluated the effects of polyvalent metal ions on the permeability of CPFX, a typical NQ, across Caco-2 cell monolayers. The \( P_{app} \) value for CPFX obtained in this study was 1.52–1.95 \( \times 10^{-6} \text{cm/s} \), consistent with a previous report (0.42–1.82 \( \times 10^{-6} \text{cm/s} \)). When Al\(^{3+} \) or Mg\(^{2+} \) was added to the solution at the concentration considered to be enough to form a chelate, permeability of CPFX was decreased in a dose-dependent manner but remained at 30% or 60% of that without metal cations, respectively. Therefore, the permeability of CPFX across intestinal epithelial cells is not fully impaired even under a high concentration of Al\(^{3+} \) or Mg\(^{2+} \). One of the possible reasons for this incomplete suppression of CPFX permeability by metal cations is the incomplete formation of a chelate. In other words, 30% of CPFX in the solution may have remained unchelated so that the free CPFX permeated the monolayer even under a high concentration of Al\(^{3+} \). However, the stoichiometry of chelation between NQs and cations ranges from 1:1 to 3:1 for Al\(^{3+} \) and from 1:1 to 2:1 for Mg\(^{2+} \), so that the amount of polyvalent metal ions was large enough to form a chelate almost completely (Fig. 1). Therefore, it is unlikely that as much as 30% of CPFX remained unchelated in the presence of a high concentration of Al\(^{3+} \). In regard to Mg\(^{2+} \), the concentration range of Mg\(^{2+} \) in this study was settled based on the concentration conceivably attained in the GI tract after the administration of a standard dose of Mg\(^{2+} \) as an antacid. Therefore, we did not investigate the effect of Mg\(^{2+} \) at higher and supraphysiological concentrations, although the concentration range is almost a physiological concentration or concentration in the physiological buffer commonly used. In any case, the concentration of Mg\(^{2+} \) investigated is considered to be high enough to form a chelate with CPFX so that chelation with Mg\(^{2+} \) again cannot fully explain the decrease in AUC clinically observed, as in the case of chelation with Al\(^{3+} \). Another possible explanation for this observation is that even chelated with metal cations CPFX can still permeate the monolayer to some extent. Although no study has directly shown that the chelate permeates across the cell monolayer, an in situ perfusion study using rat duodenum and jejunum showed that the absorption of levofloxacin (LVFX) was not affected by the presence of Al\(^{3+} \) at a concentration equal to that of LVFX. Therefore, it remains possible that CPFX permeated the cell monolayer even under the condition where chelate was formed. We calculated the free fraction of CPFX by using the binding constant of CPFX and Al\(^{3+} \) (8.434 \( \times 10^5 \text{M}^{-1} \)) reported previously. As a result, the non-chelated fractions were estimated to be 68.3%, 39.3%, 18.0%, 5.3%, and 1.7% in the presence of 0.1, 0.2, 0.3, 0.5, and 1 mM Al\(^{3+} \), respectively. These unbound fractions are smaller than the remaining permeability in the presence of the respective Al\(^{3+} \) concentration (Fig. 1), again suggesting that not only free CPFX but also the chelated CPFX is accountable for the \( P_{app} \). On the other hand, certain NQs are reported to be transported by OATP1A2, and Caco-2 cells are reported to express mRNA of OATP1A2. Therefore, permeation of non-chelated CPFX is possibly mediated by OATP1A2. However, transporters such as OATPs are quite substrate-specific so that it is unlikely that chelated CPFX is also transported by OATPs in the same way as free CPFX. In any case, it remains unclear whether CPFX permeates across the cell monolayer alone or along with Al\(^{3+} \). However, taking into account the finding in the in vitro study using rat jejunum that the concentration of Al\(^{3+} \) in the serosal side remained unchanged even after spiking CPFX and Al\(^{3+} \) together into the mucosal side, Al\(^{3+} \) may not permeate along with CPFX even if CPFX permeates under the condition where chelate was formed. Indeed, both Al\(^{3+} \) and Mg\(^{2+} \) obviously decreased the permeation of CPFX across Caco-2 monolayers. Qualitatively, this finding is consistent with the clinical observation. However, from the quantitative viewpoint, the remaining \( P_{app} \) of CPFX permeation, i.e., up to 30% or 60% by Al\(^{3+} \) or Mg\(^{2+} \), cannot explain the decrease in AUC reported in vivo, i.e., the decrease to 4.3% and 20.9% of the control by concomitant administration of Al\(^{3+} \) and Mg\(^{2+} \), respectively.

We also evaluated quantitatively the adsorption of CPFX to polyvalent metal cation product (Alumigel or Maglax) under two pH conditions. As a result, the addition of Alumigel or Maglax did not decrease the concentration of CPFX in the supernatant of Solution I (pH 1.2), suggesting that Alumigel and Maglax do not likely exert physicochemical adsorption in the stomach. By contrast, CPFX only partially dissolved into Solution II (pH 6.8) in the absence of metal cations with a concentration of 0.434 mM in the supernatant, which is consistent with the expected solubility of CPFX: 0.726 mM and 0.275 mM in pH 6 and 7, respectively. Therefore, CPFX is considered to be almost saturated in Solution II under current experimental conditions. However, in spite of the lack of significant change in pH after the addition of Alumigel, CPFX concentration in the supernatant increased in an amount of Alumigel-dependent manner. Breda et al. have previously reported that the Al\(^{3+} \) ion increases the solubility of CPFX at a pH range from 1 to 8 conceivably by forming a chelate. Therefore, the increase of CPFX concentration in the supernatant by Alumigel in this study may be attributable to the solubilization by chelation with Al\(^{3+} \). We used citrate buffer because phosphate buffer resulted in precipitation of CPFX. Therefore, the citrate ion may have possibly increased the solubility and altered the physicochemical adsorption of CPFX. In other words, the type of anion may affect the adsorption kinetics of CPFX. In any case, no decrease in the concentration of CPFX by possible adsorption to Alumigel was detected in this study, and physicochemical adsorption of CPFX on Alumigel is again unlikely in the intestine. On the other hand, Maglax decreased the concentration of CPFX in the supernatant of Solution II, along with the increase in pH in an amount of Maglax-dependent manner. As the solubility of CPFX becomes minimum at pH 8, the decreased solubility of CPFX may be attributable to the increase in pH by Maglax. However, the solubility of CPFX increases under pH > 8. In this study, even at a pH of more than 8, CPFX concentration in the supernatant failed to recover, implying that the increase in pH change by Maglax cannot explain the decrease in CPFX concentration in the supernatant at pH > 8. Arayne et al. have previously demonstrated that antacids, such as aluminum hydroxide and magnesium hydroxide, decreased the concentration of CPFX in the supernatant at pH 9 during a dissolution kinetics study and concluded that the decrease is attributable to the adsorption of CPFX to antacid but not explained by the change in pH. The above being taken together, it is likely that physicochemical adsorption of CPFX on Maglax occurs only under the non-physiological basic condition of pH > 8.

The influences on CPFX solubility in Solution II (intestinal condition) were distinct between two cations, aluminum and magnesium. The solubilities of the chelate of LVFX and polyvalent metal cations are reported to vary among cations: Cu\(^{2+} \) > Al\(^{3+} \) >
Fe$^{2+}$ > Mg$^{2+}$ > Zn$^{2+}$ > Ca$^{2+}$. In our study, Alumigel® solubilized CPFX much more than Maglax® as well. Therefore, it is highly conceivable that the solubility of CPFX also varies among polyvalent metal cations. However, current observation in the adsorption study is not consistent with the results of clinical studies. As described above, the AUC of CPFX was decreased by an antacid containing Al$^{3+}$ while the solubility of CPFX in the presence of 10 mM Alumigel® or Maglax® was 37.8% and 7.75% of CPFX (% of added concentration), respectively. Taken altogether, neither physicochemical adsorption nor change in CPFX solubility by pH increase could explain the interaction between CPFX and antacids containing Al$^{3+}$ or Mg$^{2+}$.

In conclusion, three possible mechanisms, i.e., the decrease in the permeability of CPFX across the intestinal epithelial cell monolayer by chelation, adsorption of CPFX to polyvalent metal cations, and the change in CPFX solubility by pH increase, could partially but not fully quantitatively explain the extent of DDI observed in the clinical studies. Further study for other mechanisms will be needed to fully explain the extent of DDIs between NQs and polyvalent metal cations.

References