Original scientific paper

Effect of divalent and trivalent metal ions on artificial membrane permeation of fluoroquinolones[1]

Nanami Nakatani, Kiyohiko Sugano*

Molecular Pharmaceutics Lab., College of Pharmaceutical Sciences, Ritsumeikan University, 1-1-1, Noji-higashi, Kusatsu, Shiga 525-8577, Japan

*Corresponding Author: E-mail: suganok@fc.ritsumei.ac.jp; Tel.: +81-77-561-2773

Abstract

The purpose of the present study was to evaluate the predictability of PAMPA for the effect of metal ions on the bioavailability of fluoroquinolones (FQ). Eleven FQs and seven metal ions were employed in this study. The PAMPA membrane consisted of a 10 % soybean lecithin (SL) – decane solution. A drug solution in MES buffer with or without a metal ion (added as a chloride salt) was added to the donor compartment. In the absence of metal ions, FQ showed relatively high permeability ($> 5 \times 10^{-6}$ cm/sec) in SL-PAMPA despite their hydrophilic and zwitterionic properties. As the PAMPA permeability ratio with/without metal ions became smaller, the urinary excretion and AUC ratios tended to be smaller, suggesting that SL-PAMPA is a suitable in vitro model to evaluate the potential effect of metal ions on the bioavailability of FQ. However, the reduction in AUC and urinary excretion was overestimated for low solubility metal ion formulations (dried aluminum hydroxide gel and $La_2(CO_3)_3 \cdot 8H_2O$). In such cases, the dissolution of the metal ion formulations and the permeation of FQs should be simultaneously evaluated.

Keywords

artificial membrane; permeability; phospholipid; fluoroquinolone; metal; cation

Introduction

Multivalent metal cations are present in many pharmaceutical and nutraceutical products, such as antacids, phosphate binders, and iron supplements. The concomitant administration of these multivalent metal salt formulations can reduce the bioavailability of various drugs, such as fluoroquinolones (FQ) [1,2], tetracyclines [3], and HIV-integrase inhibitors [4]. In an acidic environment of the stomach, a metal cation exists as a dissociated ion. However, it could form a complex with a drug molecule in a neutral pH environment of the small intestine. It is well known that multivalent metal cations reduce the intestinal membrane permeation of some FQs by chelate formation [2]. To avoid FQ - multivalent cation interactions in the gastrointestinal tract, it is recommended to separate the drug administration times [5]. However, this may decrease patient medication adherence. Currently, more than a dozen FQs and several multivalent metal

---

[1] This paper is dedicated to the memory of Dr. Konstantin Tsinman

salt formulations are prescribed in clinical practice. However, it is practically impossible to examine the effect of metal salt formulations on the bioavailability of FQs for all combinations by clinical studies.

The parallel artificial membrane permeation assay (PAMPA) has been widely used to assess the passive membrane permeation of drugs [6–9]. Recently, we reported that the membrane permeation of tetracyclines and the effect of metal ions can be evaluated using a phospholipid-based PAMPA [10,11]. However, it has been unknown whether PAMPA can predict the effect of metal salt formulations on the bioavailability of FQs.

The purpose of the present study was to evaluate the predictability of PAMPA for the effect of metal ions on the bioavailability of FQs. Eleven FQs and seven metal ions are employed in this study (Figure 1, Table 1).
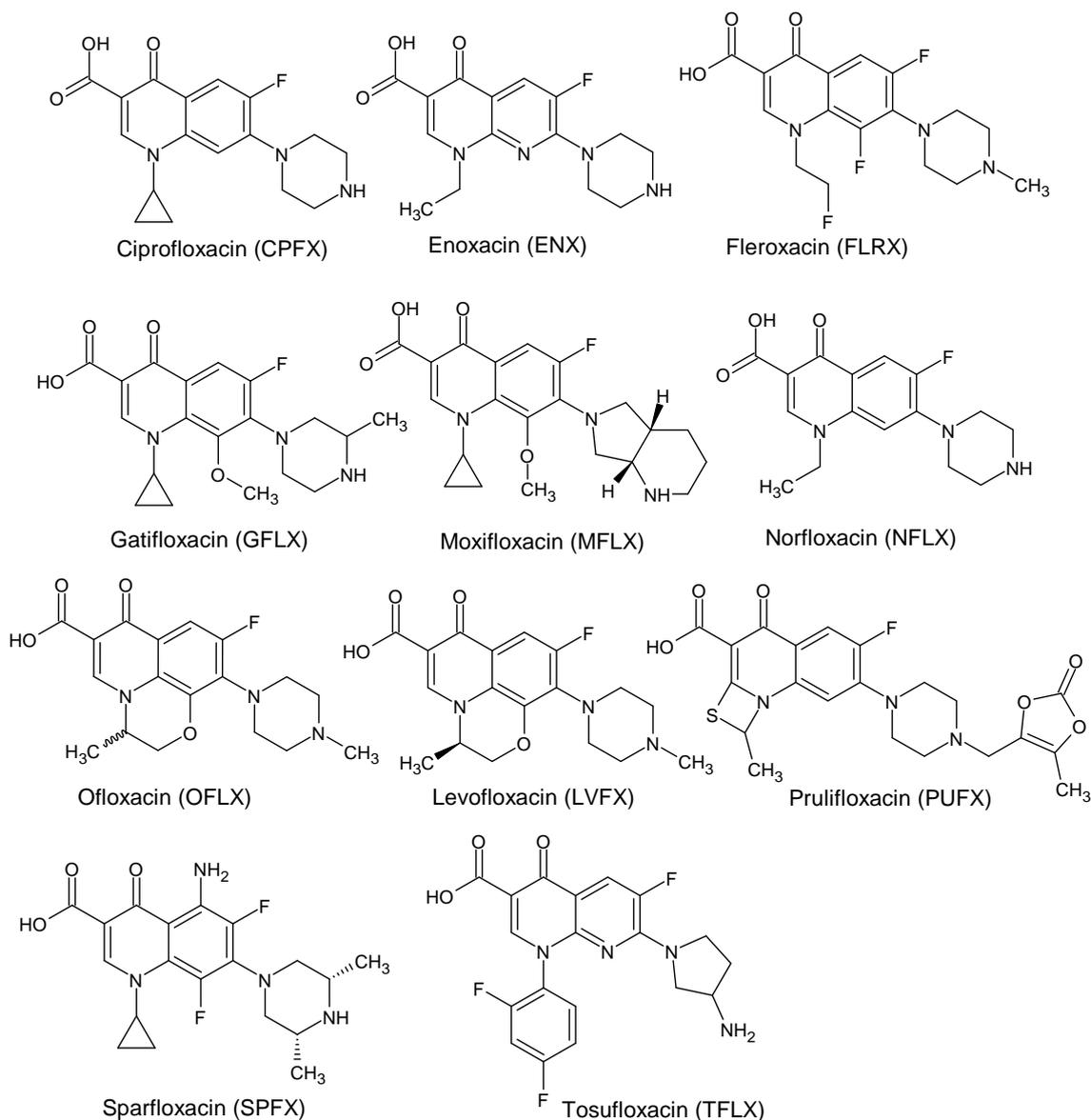
Figure 1. Chemical structures of fluoroquinolones used in this study

Experimental

Materials

Norfloxacin (NFLX), ofloxacin (OFLX), tosufloxacin tosylate monohydrate (TFLX), calcium dichloride, magnesium dichloride, iron(II) dichloride tetrahydrate, iron(III) chloride hexahydrate, aluminum(III) chloride hexahydrate, zinc chloride, lanthanum chloride heptahydrate, 1-octanol, sodium dihydrogen phosphate dihydrate, sodium chloride, sodium hydroxide, decane, 8 M NaOH, and 0.1 vol% trifluoroacetic acid-

acetonitrile were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Ciprofloxacin hydrochloride monohydrate (CPFX), levofloxacin (LVFX), enoxacin sesquihydrate (ENX), fleroxacin (FLRX), and prulifloxacin (PUFX) were purchased from TCI (Tokyo, Japan). Gatifloxacin (GFLX) and sparfloxacin (SPFX) were purchased from Funakoshi Co., Ltd (Tokyo, Japan). Moxifloxacin (MFLX) was purchased from Combi-Blocks Inc. (San Diego, USA). 2-Morpholinoethanesulfonic acid (MES) was purchased from Dojindo laboratories (Tokyo, Japan). 0.1 vol% trifluoroacetic acid-distilled water was purchased from Kanto chemical co., inc (Tokyo, Japan). Soybean lecithin was provided by Tsuji Oil Mills co., Ltd (Mie, Japan) (SLP-White, phosphatidylcholine (24-32 %), phosphatidylethanolamine (20-28 %), phosphatidylinositol (12-20 %), phosphatidic acid (8-15 %), and lysophosphatidylcholines (1-5 %) (based on the product information provided by the manufacturer)).

Table 1. Physicochemical properties of fluoroquinolones used in this study

Drug	MW	pK_a	log D (pH 6.5)[a]	UV (nm)	pK_a Ref.
Ciprofloxacin (CPFX)	331	6.1, 8.7	-1.38 ± 0.15	335	[12]
Enoxacin (ENX)	320	6.3, 8.7	-1.20 ± 0.39	330	[12]
Fleroxacin (FLRX)	369	5.5, 8.1	-0.55 ± 0.10	283	[12]
Gatifloxacin (GFLX)	375	5.7, 8.7	-0.97 ± 0.04	330	[13]
Levofloxacin (LVFX)	361	6.1, 8.2	-0.56 ± 0.11	335	[14]
Moxifloxacin (MFLX)	401	6.4, 9.5	-0.72 ± 0.38	350	[12]
Norfloxacin (NFLX)	319	6.3, 8.4	-1.35 ± 0.17	280	[12]
Ofloxacin (OFLX)	361	6.1, 8.2	-0.55 ± 0.12	330	[12]
Prulifloxacin (PUFX)	461	5.6, 6.3	0.86 ± 0.02	283	[15]
Sparfloxacin (SPFX)	392	6.3, 8.8	-0.26 ± 0.03	300	[12]
Tosufloxacin (TFLX)	404	5.8, 8.7	-0.14 ± 0.10	270	[16]

[a] Measured in this study. Mean ± SD (N = 3)

Methods

PAMPA assay

The PAMPA sandwich consisted of a 96-well filter plate (hydrophobic PVDF, 0.45 μm) and a PAMPA acceptor plate (Merck Millipore, MA, USA). The acceptor plate was filled with 300 μL of a 50 mM MES buffer solution (pH 6.5). The filter bottom of the donor well was coated with 5 μL of a 10 % soybean lecithin (SL) – decane solution prepared in our laboratory [11]. The soybean lecithin–decane membrane (SL–PAMPA) was used because it most likely mimics the intestinal membrane [17].

A drug solution (0.5 mM in pH 6.5 50 mM MES buffer, 200 μL) with or without a metal ion was added to the donor compartment. The PAMPA sandwich was then incubated at 37 °C for 3 h without stirring. After incubation, 150 μL of both the donor and acceptor solutions were transferred to a UV plate. The concentrations of FQs were measured by UV absorption except for TFLX (Table 1). The concentration of TFLX was measured by HPLC (LC-20AD, Shimazu Corporation, Kyoto, Japan) (Column: ZORBAX Eclipse Plus C18 Narrow Bore RR 2.1×50 mm column with 3.5 μm particles (Agilent Technologies, CA); flow rate: 0.60 mL/min; mobile phase: 20 % 0.1 vol% trifluoroacetic acid-acetonitrile, 80 % 0.1 vol% trifluoroacetic acid-distilled water (isocratic elution); detection wavelength: 270 nm; column temperature: 40 °C ; injection volume: 10 μL). The PAMPA permeability (P_e) was measured in triplicate. The P_e value was calculated by the following equations [17].

$$P_e = -\frac{2.303 V_D}{A \cdot t} \left(\frac{1}{1+r_v}\right) log \left(-r_v + \left(\frac{1+r_v}{1-R}\right)\frac{C_D(t)}{C_D(0)}\right) \tag{1}$$

$$R = 1 - \frac{C_D(t)}{C_D(0)} - \frac{1}{r_v}\frac{C_A(t)}{C_D(0)} \tag{2}$$

$$r_V = \frac{V_D}{V_A} \tag{3}$$

where P_e is the effective permeation coefficient (cm/s), A is the filter surface area (0.266 cm^2), V_D and V_A are the volumes (mL) of the donor and acceptor phases, respectively, t is the incubation time, $C_D(t)$ is the concentration of a drug in the donor phase at time t, R is the membrane retention factor, and r_v is the volume ratio. These equations are generally used to calculate P_e for PAMPA in the literature. The details for the derivation of these equations have been reported elsewhere [17]. In Equations 1 to 3, the mass balance of a drug in the donor fluid, the acceptor fluid, and the membrane are considered. The R values ranged from < 0.1 (CPFX, ENX, NFLX, OFLX, LVFX) to 0.36 (TFLX) in this study. Because it is difficult to collect samples multiple times from a 96-well plate, PAMPA P_e is usually calculated from one-time point data. In typical PAMPA experiments, the steady state is achieved within 0 to 45 min [17]. Therefore, PAMPA P_e is usually calculated from one-time point data at > 2 h [6–9].

Log D measurement of the fluoroquinolones

The octanol-buffer distribution coefficient (log D) was determined at pH 6.5 by a shake-flask method. The octanol and buffer phases were mutually pre-saturated before use. A buffer solution of a model drug (1.0 mM, 0.5 mL, 50 mM sodium – phosphate buffer) and octanol (2.5 mL) was added to a 15 mL tube. The sample was vigorously shaken for 90 min at 25 °C. Because log D is a physicochemical property of a drug, it was measured at a standard condition of 25 °C, rather than 37 °C. The concentration of FQs in the aqueous phase was determined as described above. Log D was measured in triplicate.

Results

Effect of physicochemical properties of fluoroquinolones on PAMPA permeability

All PAMPA permeability data are summarized in the supplemental material (Table S1). The effect of the physicochemical properties of FQs on PAMPA P_e was first investigated (Figure 2). The P_e value tended to increase as log D increased. Above log D > 0, the P_e value reached a plateau value of about 30 × 10^{-6} cm/s. There was no correlation between P_e and the pK_a values.
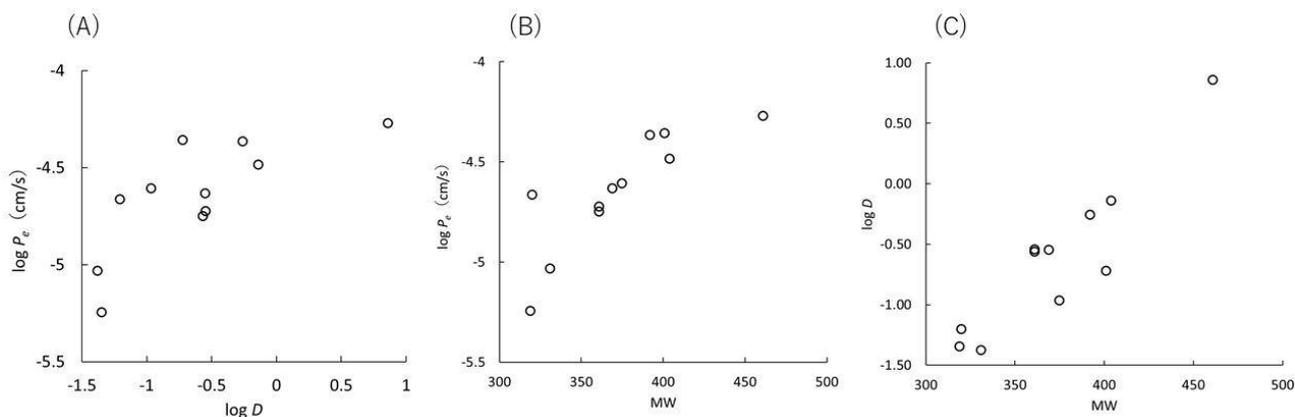
Figure 2. Correlation between PAMPA permeability, log D, and MW

In vitro – in vivo correlation of FQ – metal ion interaction

Since metal ions can interact with phosphate and citrate ions, MES buffer was used in this study. The concentration of metal ions was set to reflect the clinical dose and the gastrointestinal fluid volume. In the presence of metal ions, the P_e values were reduced in all FQs (supplemental material Table S1). Trivalent ions (Al^{3+}, Fe^{3+}, and La^{3+}) tended to show a stronger inhibition effect than divalent ions. The correlations between the urinary excretion (Ur) and AUC ratios in humans and the PAMPA P_e ratio are shown in Figure 3 (categorized by metal ions) and also in Supplemental Material Figure S1 (categorized by FQ). Clinical and in vivo canine data are summarized in Supplemental Material Table S2 with the references. There is a general trend that as the P_e ratio (with/without metal ions) becomes smaller, the reduction in AUC and Ur becomes more significant. However, PAMPA tended to overpredict the reduction in AUC and Ur, especially for Al^{3+} and La^{3+} with high permeability FQs ($P_e > 10^{-5}$ cm/s).
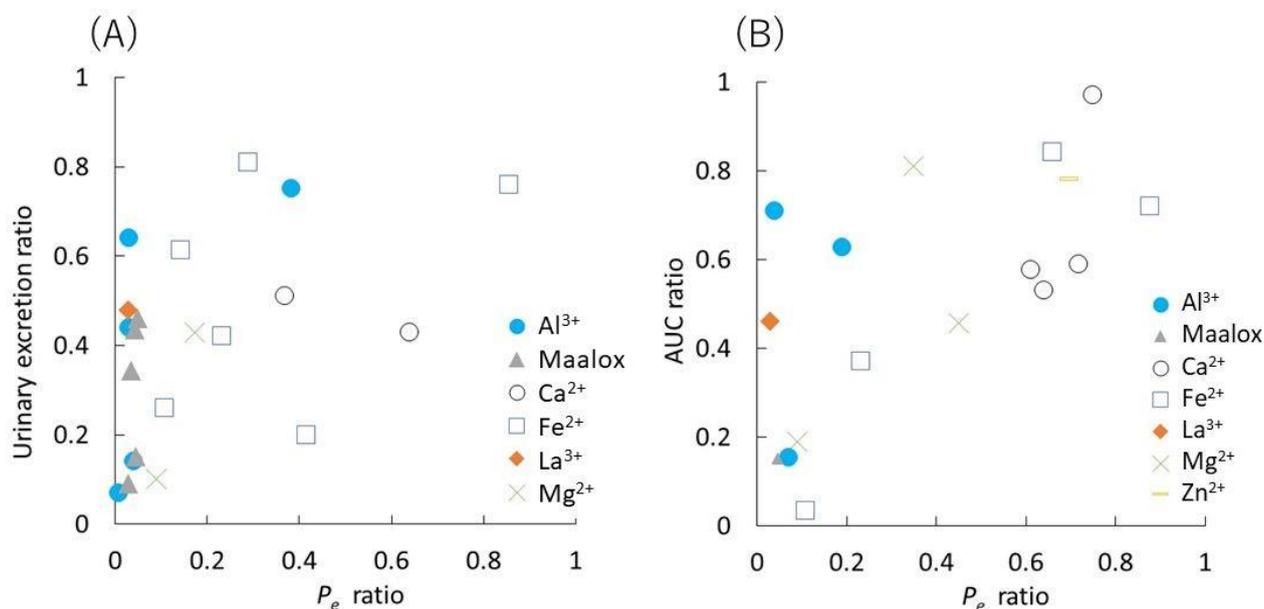
Figure 3. Correlation between PAMPA P_e ratio with/without metal ions and (A) urinary excretion ratio and (B) AUC ratio in humans (annotated by metal ions).

The interactions between norfloxacin (NFLX) and various metal ions have been investigated in dogs [18]. There is a good correlation between the PAMPA P_e ratio and the in vivo C_{max} and AUC ratios (Figure 4).
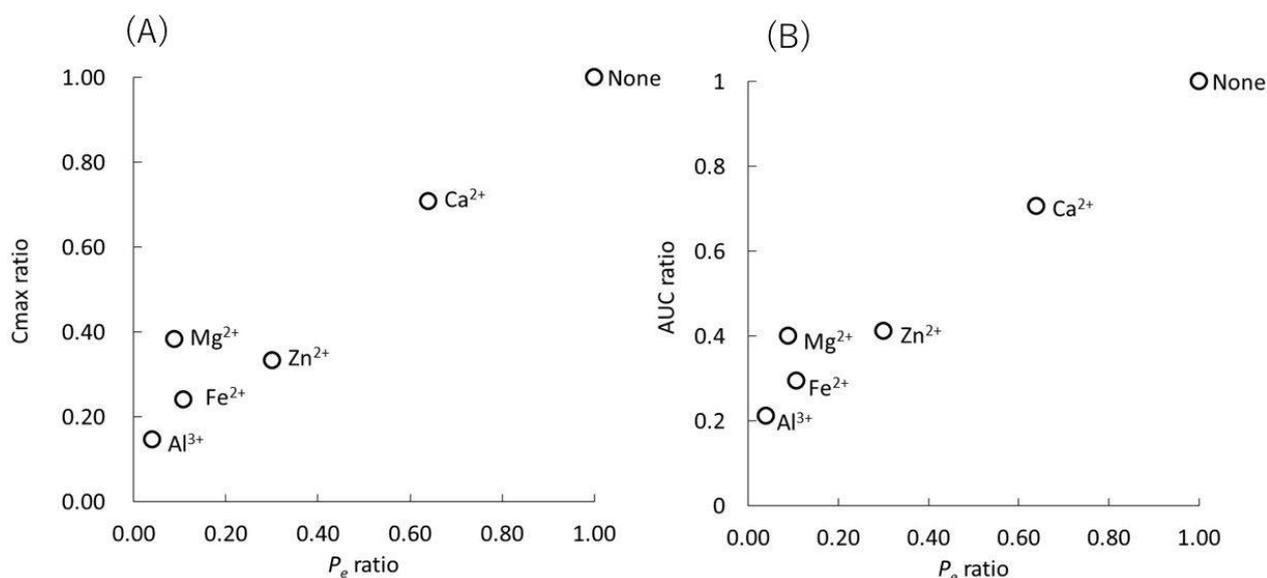
Figure 4. Correlation between the PAMPA P_e ratio and (A) C_{max} and (B) AUC ratios in dogs.

Discussion

At pH 6.5, FQ molecules exist as a zwitterion (Table 1). According to the pH-partition hypothesis, zwitterionic drugs are often assumed to show poor membrane permeability [19]. However, we previously reported that zwitterionic tetracyclines can permeate the PAMPA membrane in the presence of phospholipids (soybean lecithin) (SL-PAMPA), despite their hydrophilic and zwitterionic properties [11]. In addition, SL-PAMPA has been used to investigate the effect of metal ions on the permeation of tetracyclines [10]. Therefore, SL-PAMPA was used in this study.

In the absence of metal ions, FQs showed relatively high permeability (> 5×10^{-6} cm/sec) in SL-PAMPA despite their hydrophilic and zwitterionic properties. The high P_e values of FQs in SL-PAMPA are in good agreement with that these FQs show good oral bioavailability. Even in the case of most hydrophilic FQs, NFLX (log D = - 1.35) and CPFX (log D = - 1.38), the bioavailability is moderate and high (> 38 % and 83 %, respectively) [20,21]. These results suggested that SL-PAMPA is suitable to assess the effect of metal ions on the intestinal membrane permeation of zwitterionic drugs. The P_e values of NFLX and CPFX are about 10 times higher than previously reported values in pION's PAMPA, which uses a 20 % dodecane solution of a lecithin mixture [19,22]. As log D was increased, the P_e value was increased until reaching the plateau value (about 30×10^{-6} cm/s at log D > 0) (Figure 2A). This plateau value would be attributed to the unstirred water layer adjacent to the PAMPA membrane [23].

As shown in Figure 2B, PAMPA P_e was also positively correlated with MW. The observed correlation between log P_e and MW may be a mere coincidence due to the choice of FQs, but it may also be explained as follows. According to the solubility-diffusion theory [24,25], partition and diffusion processes determine the lipid membrane permeation of a drug. As MW increases, the diffusion coefficient decreases. On the other hand, the partition process depends on the lipophilicity of a drug. The lipophilicity of a drug is determined by the balance of drug-solvent interactions (e.g., hydrogen bonding) and the cavity effect, the latter of which is proportional to MW. Therefore, when drug-solvent interaction is similar (like among FQ series), log D increase with MW (Figure 2C). In most cases of drug membrane permeation, the influence of a partition process is greater than that of a diffusion process. It is well known that the membrane permeability of drugs correlates with their lipophilicity [9]. Therefore, even though an increase in MW decreases the diffusion process, P_e can show a positive correlation with MW. More detailed analysis is required to further investigate this point, such as using Abraham's solute descriptors [26].

In this study, metal ions are added as chloride salts because they are soluble in aqueous media. The P_e ratio of NFLX was in good agreement with the C_{max} and AUC ratios observed in dogs (Figure 4) [18]. In that study, NFLX was administered as a 0.01 N HCl solution with the metal chloride salts. Therefore, the dissolution process of FQs and multivalent metal salt formulations were neglected. The effect of Mg^{2+} in dogs was slightly overestimated by PAMPA. The reason for this was not clear.

However, in clinical cases where these formulations are administered as solid dosage forms, PAMPA tended to overpredict the reduction of AUC and C_{max}, especially for Al^{3+} and La^{3+} formulations (Figure 3). In clinical formulations, Al^{3+} is contained as dried aluminum hydroxide gel and La^{3+} is contained as $La_2(CO_3)_3 \cdot 8H_2O$. Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} are also formulated as the other solid forms ($CaCO_3$, MgO, etc.). Some of these solid forms would dissolve slowly in the intestinal fluid so FQs can be absorbed before metal ions are released from the formulations, especially for high permeability FQs. For example, the dissolution of $La_2(CO_3)_3 \cdot 8H_2O$ is about 50 % at 15 min in the gastric environment of pH 1.2 and there is little dissolution in the intestinal environment of pH 6.8 [27]. For more quantitative prediction, the dissolution of a metal ion formulation and the permeation of an FQ should be simultaneously evaluated.

The detailed analysis of equilibria between FQs and metal ions is out of the scope of this study, as it has been reported for some of the FQs [28]. Previously, Wallis et al. reported that the formation constant of the NFLX – metal ion complex correlates with in vivo bioavailability in dogs [18]. The chelate formation constant (log unit) and the percentage of NFLX complexed at pH 6.5 are reported to be 2.2 (8.5 %), 3.0 (35 %), 3.8 (81 %), 4.0 (78 %), and 7.0 (99 %) for Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, and Al^{3+}, respectively. In the present study, the trivalent metal ions (Al^{3+}, Fe^{3+}, La^{3+}) reduced P_e more than the divalent metal ions. Among the divalent metal ions, no general trend was observed, except that Mg^{2+} reduced P_e more than Ca^{2+} in all FQs. This trend was not observed in tetracyclines [10].

Conclusion

In conclusion, the P_e values of FQs are relatively high in SL-PAMPA. As the P_e ratio (with/without metal ions) becomes smaller, the reduction in AUC and Ur tended to be more significant. However, the reduction in AUC and Ur is overestimated for low solubility metal ion formulations (especially Al^{3+} and La^{3+} formulations). In such cases, the dissolution of a metal ion formulation and the permeation of an FQ should be simultaneously evaluated for more quantitative prediction by an in vitro experiment. SL-PAMPA is suitable to evaluate the potential effect of metal salt formulations on the bioavailability of FQs.

Conflict of interest: Authors declare no conflict of interest.

References

[1] A. Stojković, L. Tajber, K.J. Paluch, Z. Djurić, J. Parojčić, O.I. Corrigan. Biopharmaceutical characterisation of ciprofloxacin-metallic ion interactions: Comparative study into the effect of aluminium, calcium, zinc and iron on drug solubility and dissolution. *Acta Pharm*. **64** (2014) 77–88. https://doi.org/10.2478/acph-2014-0007.

[2] Ž. Simon, B. Katja, U. Darko, V. Marjan, K. Albin. Metal cation-fluoroquinolone complexes do not permeate through the intestinal absorption barrier. *J. Pharm. Biomed. Anal*. **53** (2010) 655–659. https://doi.org/10.1016/j.jpba.2010.05.021.

[3] J.T. Doluisio, A.N. Martin. Metal complexation of the tetracycline hydrochlorides. *J. Med. Chem*. **6** (1963) 16–20. https://doi.org/10.1021/jm00337a003.

[4] R. Krishna, L. East, P. Larson, C. Valiathan, K. Butterfield, Y. Teng, M. Hernandez-Illas. Effect of metal-cation antacids on the pharmacokinetics of 1200 mg raltegravir. *J. Pharm. Pharmacol.* **68** (2016) 1359–1365. https://doi.org/10.1111/jphp.12632.

[5] S.K. Pitman, U.T.P. Hoang, C.H. Wi, M. Alsheikh, D.A. Hiner, K.M. Percival. Revisiting oral fluoroquinolone and multivalent cation drug-drug interactions: Are they still relevant? *Antibiotics*. **8** (2019). https://doi.org/10.3390/antibiotics8030108.

[6] M. Kansy, F. Senner, K. Gubernator. Physicochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes. *J. Med. Chem.*, **41** (1998), 1007-1010. https://doi.org/10.1021/jm970530e.

[7] A. Avdeef, S. Bendels, L. Di, B. Faller, M. Kansy, K. Sugano, Y. Yamauchi. PAMPA - Critical factors for better predictions of absorption. *J. Pharm. Sci*. **96** (2007). https://doi.org/10.1002/jps.21068.

[8] L. Di, P. Artursson, A. Avdeef, L.Z. Benet, J.B. Houston, M. Kansy, E.H. Kerns, H. Lennernäs, D.A. Smith, K. Sugano. The Critical Role of Passive Permeability in Designing Successful Drugs. *ChemMedChem*. (2020) 1862-1874. https://doi.org/10.1002/cmdc.202000419.

[9] K. Sugano, M. Kansy, P. Artursson, A. Avdeef, S. Bendels, L. Di, G.F. Ecker, B. Faller, H. Fischer, G. Gerebtzoff, H. Lennernaes, F. Senner. Coexistence of passive and carrier-mediated processes in drug transport. *Nat. Rev. Drug Discov*. **9** (2010) 597-614. https://doi.org/10.1038/nrd3187.

[10] S. Yamauchi, D. Inoue, K. Sugano. Permeation characteristics of tetracyclines in parallel artificial membrane permeation assay II: Effect of divalent metal ions and mucin. *ADMET DMPK* **8** (2020) 129-138. https://doi.org/10.5599/admet.797.

[11] S. Yamauchi, K. Sugano. Permeation characteristics of tetracyclines in parallel artificial membrane permeation assay. *ADMET DMPK* **7** (2019) 151–160. https://doi.org/10.5599/admet.657.

[12] F. Sörgel, J. Bulitta, M. Kinzig-Schippers. Pharmakokinetik der Chinolone. *Pharmaziein Unserer Zeit*. (2001) 418–427. https://doi.org/10.1002/1615-1003(200109)30:5<418::AID-PAUZ418>3.0.CO;2-%23 (accessed August 24, 2022).

[13] M. Caianelo, C. Rodrigues-Silva, M.G. Maniero, J.R. Guimarães. Antimicrobial activity against Gram-positive and Gram-negative bacteria during gatifloxacin degradation by hydroxyl radicals. *Environ. Sci. Pol. Res*. **24** (2017) 6288-6298. https://doi.org/10.1007/s11356-016-6972-y.

[14] Levofloxacin Hydrate Drug Product Information (CRAVIT TABLETS), Daiichi Sankyo Company, https://www.info.pmda.go.jp/go/pack/6241013C2024_1_21 (accessed August 24, 2022).

[15] P. Ravisankar, Ch.V.P. Rao, N.A. Qureshi, H.A. Alsubaie, M.K. Alharbi, G.I.M. Ali, S.M. Alsanad, S.A. Ali, S. Mahanand, S.W.` Khan, K.T. Magalhães-Guedes, J.I. Druzian, R.F. Schwan, I.L. Nunes, J.A.V. Costa, T. Attard, E. Attard, L.M. Shengo, T.H. Mundongo, I.U. Umoh, A.U. Ekanem, S.U. Rehman, H.M. Rafique, F.A. Qureshi, S. Shahid, H.-K. Kang, H.-J. Kim, J.-S. Kang, I. Dey, T.K. Das, C. Roy, M.K. Pillai, S. Magama, L.I. Santi, N. Katsuto, S. Jin-Ichi, M.S.T. Sheikuduman, B. Mangla, A. Jain, V.R. Salunkhe. Trends in Pharmaceutical Research and Development Vol. 1, Book Publisher International (a part of SCIENCEDOMAIN International), 2020. https://doi.org/10.9734/bpi/tprd/v1.

[16] Tosufloxacin tosylate Drug Product Information (Ozex tablets), FUJIFILM Toyama Chemical Co. Ltd. https://www.info.pmda.go.jp/go/pack/6241010F1020_2_06/ (accessed August 24, 2022).

[17] A. Avdeef. Absorption and Drug Development: Solubility, Permeability, and Charge State, Second Edition, John Wiley & Sons, Inc., 2012. https://doi.org/10.1002/9781118286067.

[18] S.C. Wallis, B.G. Charles, L.R. Gahan, L.J. Filippich, M.G. Bredhauer, P.A. Duckworth. Interaction of norfloxacin with divalent and trivalent pharmaceutical cations. In vitro complexation and in vivo pharmacokinetic studies in the dog. *J. Pharm. Sci*. **85** (1996) 803–809. https://doi.org/10.1021/js960087f.

[19] K.Y. Tam, A. Avdeef, O. Tsinman, N. Sun. The permeation of amphoteric drugs through artificial membranes - An in combo absorption model based on paracellular and transmembrane permeability. *J. Med. Chem*. **53** (2010) 392–401. https://doi.org/10.1021/jm901421c.

[20] N. Campbell, M. Kara, B. Hasinoff, W. Haddara, D. McKay. Norfloxacin interaction with antacids and minerals. *Br. J. Clin. Pharmacol*. **33** (1992) 115–116. https://doi.org/10.1111/j.1365-2125.1992.tb04010.x.

[21] Ciprofloxacin HCl Drug Product Information (Ciproxan). Bayer. https://www.info.pmda.go.jp/go/pack/6241008F1023_1_23/ (accessed August 24, 2022).

[22] M. Bermejo, A. Avdeef, A. Ruiz, R. Nalda, J.A. Ruell, O. Tsinman, I. González, C. Fernández, G. Sánchez, T.M. Garrigues, V. Merino. PAMPA - A drug absorption in vitro model: 7. Comparing rat in situ, Caco-2, and PAMPA permeability of fluoroquinolones. *Eur. J. Pharm. Sci.* **21** (2004) 429–441. https://doi.org/10.1016/j.ejps.2003.10.009.

[23] P.E. Nielsen, A. Avdeef. PAMPA - A drug absorption in vitro model: 8. Apparent filter porosity and the unstirred water layer. *Eur. J. Pharm. Sci.* **22** (2004) 33–41. https://doi.org/10.1016/j.ejps.2004.02.003.

[24] T.X. Xiang, Y.H. Xu, B.D. Anderson. The barrier domain for solute permeation varies with lipid bilayer phase structure. *J. Membr. Biol.* 165 (1998) 77-90. https://doi.org/10.1007/s002329900422.

[25] J.M. Diamond, Y. Katz. Interpretation of nonelectrolyte partition coefficients between dimyristoyl lecithin and water. *J. Membr. Biol.* 17 (1974) 121-154. https://doi.org/10.1007/BF01870176.

[26] M.H. Abraham. Scales of solute hydrogen-bonding: Their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* 22 (1993) 73-83. https://doi.org/10.1039/CS9932200073.

[27] Lanthanum Carbonate Granules Drug Product Information. NIPRO CORPORATION. https://med.nipro.co.jp/servlet/servlet.FileDownload?file=00P2x00000BcDPtEAN (accessed August 24, 2022).

[28] B. Urbaniak, Z.J. Kokot. Analysis of the factors that significantly influence the stability of fluoroquinolone-metal complexes. *Anal. Chim. Acta* 647 (2009) 54–59. https://doi.org/10.1016/-j.aca.2009.05.039.