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Metal cation–fluoroquinolone complexes do not permeate through the intestinal absorption barrier

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ABSTRACT

A case of a clinically significant interaction between a fluoroquinolone antimicrobial agent and metal cations was first reported in 1985. The hypothesized mechanism – decreased fluoroquinolone intestinal permeability due to complex formation between metal cations and ciprofloxacin – was based on a 1978 work with nalidixic acid. While clinical research and numerous in vitro physico-chemical and chelation chemistry studies of fluoroquinolone–metal cation combinations simply accepted this explanation, the few in vitro studies, which were aimed to investigate the nature of the interaction mechanism, provided conflicting results. This was most likely due to the sensitivity of the interaction to in vivo conditions, which were not reproduced in vitro. All the above-mentioned studies including our earlier work in vitro were performed with diluted solutions of fluoroquinolones and metal cations. Now we provide results obtained on rat intestine in side-by-side diffusion chambers with saturated solutions of fluoroquinolones and metal cations in the donor compartment as it is most likely in the human small intestine in vivo. The fluoroquinolone permeability decreased under these conditions in the presence of metal cations and the obtained results show that the ciprofloxacin–aluminum complex does not permeate the intestinal mucosal membrane.

1. Introduction

A case of a clinically significant interaction between a fluoroquinolone antimicrobial agent and metal cations present in antacids, mineral supplements and food was first reported in 1985. Höffken et al. observed a decrease of ciprofloxacin bioavailability caused by a co-administration of a magnesium and aluminum containing antacid. The authors of this first report speculated that the described drug–drug interaction may be related to the formation of complexes (coordination compounds) between fluoroquinolones and metal cations) [1]. Interestingly, it has never been established whether the fluoroquinolone–metal cation complexes are capable of penetrating the intestinal mucosal membrane!

Numerous clinical studies in which the authors investigated drug–drug interactions between fluoroquinolones and preparations containing metal cations followed. Co-administration of fluoroquinolones and preparations containing metal cations generally resulted in a gross decrease of the fluoroquinolone bioavailability—up to 90% [2]. Fluoroquinolone coordination chemistry was also intensively studied [3]. However, these studies were not aimed to reveal how the reduction of fluoroquinolone bioavailability could be a direct consequence of complexation with metal cations.

Less effort was invested into establishing the exact biopharmaceutical mechanism of the fluoroquinolone metal cation interaction. Several possibilities were studied, but the obtained results were different or even contradictory. We showed recently that the decrease of fluoroquinolone solubility does not occur at physiologic conditions in the presence of metal cation salts [4]. This is not in contrast to the findings that the solubility of complexes themselves is low, because the fluoroquinolone–metal cation complexes (usually in 1:1 and 1:2 ratios in pH neutral media) are only additional species to otherwise existing zwitterions, neutral, cationic and anionic forms of fluoroquinolone [5,6]. A hypothesis about altered stability of fluoroquinolones in the gastric acid [7] was also proven to be wrong [8]. A suggestion by Tanaka et al. that adsorption of fluoroquinolones on the metal cation hydroxides, which can precipitate in the duodenum, was interesting, but as they concluded, needed further investigation [9]. This was never performed, most likely because a simultaneous "in vitro" simulation of all the critical conditions in the gastro-intestinal tract (pH, ion strength, individual ion concentrations, protein binding, motility, absorption, etc.) that can significantly influence the precipitation is simply not feasible. Another hypothesis – formation of a micellar phase including fluoroquinolones – was suggested by Sánchez et

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al. [10], but according to an earlier research by Ross et al. [11] this is not relevant.

A formation of low-permeability coordination compounds between fluoroquinolones and metal cations with very low or zero permeability [12,2,13,3] could result in a decrease of total fluoroquinolone permeability and therefore could cause a decrease of fluoroquinolone bioavailability. One way to test this was to measure the influence of metal cations on the apparent octanol/water partition coefficients of fluoroquinolones. Ross et al. discovered that the apparent partition coefficients of lomefloxacin and fleroxacin are decreased by the presence of Ca^{2+}, Mg^{2+} and Al^{3+} at pH = 5.1–5.3 [11], while Sánchez Navarro et al. showed that the partition coefficients of ciprofloxacin and ofloxacin are not influenced by the presence of Ca^{2+} at pH = 7.4 [14]. The other way to test this hypothesis was to measure the "in vitro" or "in situ" permeability of fluoroquinolones through rat intestine or artificial membranes. Sánchez Navarro reported a reduction of fluoroquinolone permeability through rat intestine [14], while others (results of Tanaka et. al, and previous studies in our institution) observed no influence of metal cations on the permeability of fluoroquinolones [9,11,15,16].

A possible reason for the contradictory results listed above could be the applied experimental conditions (pH and/or concentrations of fluoroquinolones and metal cations), which were often not even similar to the conditions in the gastro-intestinal tract. The measurements were performed at too acidic pH or/and at too low concentrations of fluoroquinolones [9,15,16] to ensure that all components were completely dissolved in the donor solutions because they have better solubility at these generally non-physiological conditions [5]. On the other hand, an ingestion of medicines containing metal cations as active ingredients would yield concentrations in the range of 1–50 mM, which is much higher than their solubility at the small intestinal pH. Similarly, the highest single doses of ciprofloxacin, norfloxacin and ofloxacin yield calculated intestinal concentrations of 9 mM, 5 mM and 4.4 mM, respectively, if 250 ml is the intestinal dissolution volume. These values (especially for ciprofloxacin) are also much higher then their solubility at the small intestinal pH [4].

In the present study we apply a novel approach to investigate whether complexes of fluoroquinolones and metal cations permeate through the intestinal mucosa. Furthermore, we also reveal to what extent the presence of metal cations in the gastro-intestinal tract reduces the permeability of fluoroquinolones through the intestinal mucosa when measured at high (saturated) concentrations of fluoroquinolones and metal cations.

2. Materials and methods

2.1. Materials

Ciprofloxacin was purchased from Fluka (Deisenhofen, Germany). Norfloxacin and ofloxacin were supplied by Sigma–Aldrich Chemie (Steinheim, Germany). The metal cation salts of analytical grade were from Merck (Darmstadt, Germany).

2.2. "In vitro" intestinal permeability studies

Rat jejunum from male Wistar rats (250–320 g) was obtained, prepared and mounted in Easy Mount side-by-side diffusion chambers (Physiologic Instruments, San Diego, CA, USA) as previously described [17]. The experiments conform to the Law for the protection of animals (Republic of Slovenia) and the use of the tissue samples from previously sacrificed animals for experimental purposes is registered at the Veterinary Administration of the Republic of Slovenia.

Standard or modified Ringer buffer with 10 mM D-glucose or 10 mM mannitol on serosal and mucosal side of the tissue, respectively, was used as an initial incubation saline. It was previously shown that the use of a modified Ringer buffer without Ca^{2+} and Mg^{2+} on one side of the isolated tissue and the implication of phosphate-free incubation saline (necessary for testing the influence of Al^{3+}, Mg^{2+}, Ca^{2+} and Fe^{2+} at high concentrations) does not impair the viability or the integrity of the isolated intestinal tissue [17]. The tissue was kept at 37 °C during the experiments and the pH of the incubation salines was 7.5. The incubation salines for the donor (mucosal) compartment were prepared by dissolving 5 mM fluoroquinolones and/or 5 mM metal cation chlorides in 0.001 M hydrochloric acid. Then the same amount of potassium chloride as it is used in the standard Ringer buffer was added and the pH of these salines was adjusted to 7.5 by sodium bicarbonate powder. Significant precipitation of fluoroquinolones and/or metal cation salts/hydroxides occurred during this pH neutralization. The osmolarity of all modified incubation salines was adjusted by NaCl to 305 mosm. Incubation salines were oxygenated and circulated by bubbling with carbogen (95% O_2 and 5% CO_2).

The experiments were performed in consecutive 100 min long phases, during which different experimental conditions were applied. After the tissue segments were placed into the diffusion chambers, 25 min was always allowed for equilibration. To test the influence of metal cations on the permeability of fluoroquinolones, the permeability of each fluoroquinolone alone was evaluated in the first phase of the experiment. This was followed by the second phase, when the donor incubation medium contained also the tested metal cation. When the effect of ciprofloxacin on the permeability of Al^{3+} was evaluated, the experiments started with blank conditions (donor compartment was without ciprofloxacin and without Al^{3+}), after 100 min continued with 5 mM Al^{3+} in the donor compartments and then ended with a third phase with both ciprofloxacin and Al^{3+} in the donor compartments.

Samples of 250 μl were withdrawn from the acceptor (serosal) in 20 min intervals and from the donor (mucosal) compartment at 30th min and at 90th min. This volume was replaced by the appropriate fresh acceptor or donor incubation medium. The concentrations of fluoroquinolones in the samples were determined by HPLC immediately after the experiment. Samples from the donor compartment were filtered and diluted at the time of sampling. The samples for the determination of Al^{3+} concentration were taken at the end of each experimental phase and frozen until analysed.

The tissue viability and integrity were controlled throughout the experiments by monitoring the trans-tissue potential difference and the short circuit current with a multi channel voltage–current clamp (model VCC MC8, Physiologic Instruments) as described previously [17]. The trans-tissue potential difference is a reliable parameter for the determination of the tissue viability [18,19]. At the end of the experiments, the trans-tissue potential difference after the addition of D-glucose to the mucosal compartment (final concentration was 25 mM) was also measured. The absolute values of the trans-tissue potential difference higher than 1.0 mV indicated, that the viability of the rat intestinal mucosa has not been affected to such an extent that it could influence the permeability of tested substances. A change of the trans-tissue potential difference higher than 0.3 mV after the addition of D-glucose also indicated good viability. The tissue integrity was additionally evaluated by the trans-tissue electrical resistance. Its value at the time-points used for the calculation of the permeability coefficient was higher than 18 $\Omega \times cm^2$. The tissue segments, which did not present adequate viability and integrity, were excluded from further data evaluation.

2.3. Analytical procedures and data analysis

Ciprofloxacin, norfloxacin and ofloxacin concentrations were measured by HPLC (Agilent 1100 series; UV detection at 278 nm

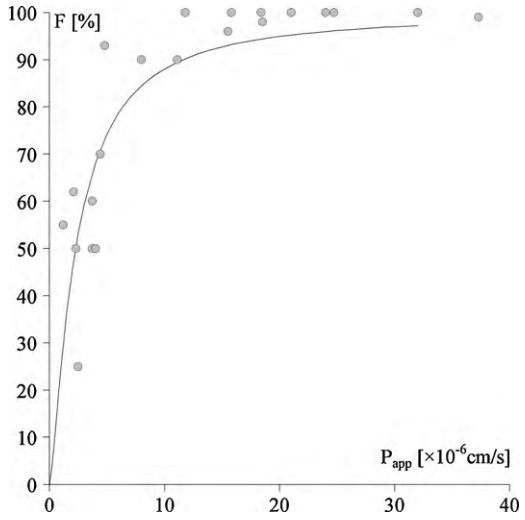

Fig. 1. Drug permeability vs. drug bioavailability data set (circles) for 21 compounds and fitted Hill curve (Eq. (2)) are presented.

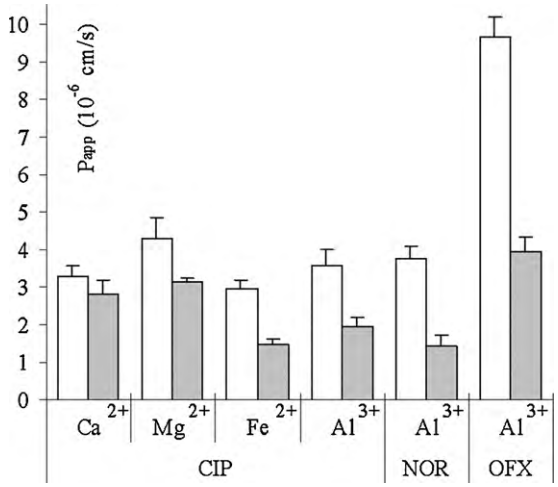

Fig. 2. Apparent permeability coefficients of three fluoroquinolones in reference experimental conditions (empty columns) and in the presence of metal cations (filled columns). CIP is ciprofloxacin, NOR is norfloxacin and OFX is ofloxacin.

(ciprofloxacin and norfloxacin) or 293 nm (ofloxacin) after separation on a 25 mm C18 Chromolith Flash column with 11.5% of acetonitrile and 88.5% of phosphate buffer (pH = 3.9). The flow rate was 3 ml/min and the injection volume was 30 μl. The retention times of ciprofloxacin and norfloxacin were 0.66 min and the retention time of ofloxacin was 0.61 min. The retention times and the peak shape were not influenced by the presence or absence of metal cations in the samples. The non-interference of metal cations on the chromatographic analysis of fluoroquinolones was also shown earlier by Kmetec at al [15]. The concentration of aluminum was determined by electrothermal atomic absorption spectrometry using a Perkin Elmer Atomic Absorption Spectrometer 1100B equipped with HGA 400 graphite furnace. Atomization was performed at 2500 °C and absorption was measured at 309.3 nm Pyrolitic coated graphite tubes with platform were applied. The sample injection volume was 20 μl. Results were evaluated using calibration curves.

The P_{app} (apparent permeability coefficient) values of investigated substances were calculated by the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0}\ [cm/s] \quad (1)$$

where dQ/dt is the steady-state appearance rate of the investigated substance on the acceptor side of the tissue, A is the exposed tissue area and C_0 is the concentration of the dissolved investigated substance measured in the filtrates of the samples taken from the donor compartment at the 30th min of the experimental phase. The dQ/dt term of the Eq. (1) is the slope of the amount-permeated vs. time plot drawn for each intestinal tissue segment used. The linearity of these plots is also evaluated. All the values of apparent permeability coefficients were calculated by the measured concentrations of the dissolved fluoroquinolones in the donor solutions/suspensions.

For the evaluation of the bioavailability changes caused by the experimentally measured changes of fluoroquinolone P_{app} values, a Hill curve (Eq. (2) and Fig. 1) was fitted to a data set of 21 compounds including 15 compounds recommended by the Center for Drug Evaluation and Research in their Guidance for Industry [20]. The data set was compiled from permeability measurements obtained in our laboratory.

$$F = \frac{F_{max} \cdot P_{app}^{\eta}}{P_{app_{50}}^{\eta} + P_{app}^{\eta}}\ [\%] \quad (2)$$

F is the "fraction absorbed" after oral drug administration, P_{app50} is the P_{app} when F equals 50% and η is the Hill coefficient. F_{max} equals 100%, P_{app50} was 3.01×10^{-6} cm/s and the value of η was 1.35.

MS Excel was used for data handling and paired t-tests. All data are given as mean ± standard error of the mean. Each mean is obtained by 4–6 measurements on different intestinal tissue segments.

3. Results and discussion

With the aim to obtain more biologically and therapeutically relevant "in vitro" permeability data regarding the absorption of fluoroquinolones after concomitant administration of drugs or food containing metal cations, we attempted to mimic the relevant conditions in the gastro intestinal tract as well as possible. Therefore, the permeability of fluoroquinolones was measured using donor suspensions with high concentrations of metal cations and fluoroquinolones instead of clear solutions which are normally used in this type of experiments. In fact, we initially completely dissolved the tested fluoroquinolones and/or metal cation salts in an acidic medium and the pH of these salines was subsequently neutralized by sodium bicarbonate powder to mimic the processes during the transition of gastric contents to the duodenum where it is neutralized. Significant precipitation of fluoroquinolones and/or metal cation salts/hydroxides occurred during the pH neutralization. This is simply due to the very low solubility of fluoroquinolones and hydroxides of the metal cations tested at the neutral pH. For example: the solubility products for $Ca(OH)_2$, $Mg(OH)_2$, $Fe(OH)_2$, $Fe(OH)_3$ and $Al(OH)_3$ are 6.5×10^{-6}, 7.1×10^{-12}, 4.1×10^{-15}, 2×10^{-39}, and 3×10^{-34}, respectively [21].

The effect of four cations (Ca^{2+}, Mg^{2+}, Fe^{2+} and Al^{3+}) on the permeability of a fluoroquinolone drug was tested with ciprofloxacin because its intestinal transport was well characterized previously [16]. The influence of highly concentrated metal cation on the permeability of three different fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) was evaluated with Al^{3+} because of its intense effect on the fluoroquinolone bioavailability [2] and because it is not transported by any active mechanism [22].

Fig. 2 shows the apparent permeability coefficients of three fluoroquinolones, the effects of different metal cations on the permeability of ciprofloxacin and the effects of aluminum cation on the permeability of norfloxacin and ofloxacin through the rat jejunum. All fluoroquinolone–metal cation combinations resulted in a reduced permeability compared to that observed when only the corresponding fluoroquinolone was present in the (mucosal)

Table 1
The "in vitro" permeability (P_{app}) of fluoroquinolones in the presence of metal cations, estimated decreases of fraction absorbed (F) and the literature [2] data for the decrease of human fluoroquinolone bioavailability after co-administration with metal cations.

Combinations tested "in vitro"	Measured P_{app} decrease (%)	Estimated[a] F decrease (%)	Bioavailability decrease		
			Clinical data from literature (%)	Reference	Metal cation source
CIP–Al^{3+}	46	36	75–91	[2,29]	Mg^{2+} and Al^{3+} antacids
CIP–Mg^{2+}	27	17			
CIP–Fe^{2+}	50	44	33–63	[2,30]	Ferrous sulfate
CIP–Ca^{2+}	14	10	40–43	[2,24]	Calcium carbonate
NOR–Al^{3+}	62	53	91	[2,23]	Mg^{2+} and Al^{3+} antacids
OFX–Al^{3+}	59	29	73	[2,31]	Mg^{2+} and Al^{3+} antacids

CIP, ciprofloxacin; NOR, norfloxacin; OFX, ofloxacin.
[a] Estimated F calculated from P_{app} with Eq. (2).

donor compartment. The differences in fluoroquinolone permeability without and with metal cations are statistically significant (paired t-tests; $\alpha = 0.05$.) in all cases except for Ca^{2+}–ciprofloxacin combination. The smaller effect of calcium on the permeability of ciprofloxacin is in accordance with the fact that calcium decreases the fluoroquinolone bioavailability much less then the other metal cations [2,23,24].

Although the effect of metal cations on the "in vitro" fluoroquinolone permeability shown in Fig. 2 is evident, the relationship between the decrease of "in vitro" permeability and "in vivo" bioavailability is not proportional [25]. Therefore a Hill curve (Eq. (2)) fitted to the human bioavailability vs. rat intestinal permeability data set of 21 compounds tested in our laboratory was applied to estimate the reduction of bioavailability caused by the measured decrease of fluoroquinolone permeability in the presence of metal cations. The estimated bioavailability reductions of tested fluoroquinolones caused by lower permeability observed in this study in the presence of applied metal cations are presented in Table 1. This table shows that most of the reductions of fluoroquinolone bioavailability estimated from the measured decreases of their P_{app} values are lower than the reduction of fluoroquinolone bioavailability observed in the clinical studies [2]. A combination of ciprofloxacin and ferrous ions resulted in an estimated bioavailability reduction within the range observed in clinical studies. Separate clinical data for interactions of fluoroquinolones with only aluminum cations or only magnesium cations are not available and data for interactions with combined aluminum–magnesium antacids are used instead for comparison. This could also contribute to the discrepancy between the estimated and clinical decreases of fluoroquinolone bioavailability (Table 1). Nevertheless, the observed results still imply that some additional mechanism may also be responsible for the decrease of fluoroquinolone bioavailability after co-administration with metal cation-containing drugs or food. This additional mechanism could include the precipitation of metal cation hydroxides in the duodenum where the acidic gastric content is neutralized by endogenous bicarbonate. Fluoroquinolones could integrate in/on this precipitate in a way which would prevent their dissolution and absorption through the intestinal mucosa. As already mentioned in the introduction of this paper, Tanaka et al. observed that "adsorption of fluoroquinolones on the solid (precipitated) metal cation hydroxides" is possible [9].

A careful review of literature shows that the hypothesis of a "formation of coordination compounds between fluoroquinolones and metal cations with very low or zero permeability" [12,2,13,3] actually originates only from a 1978 work by Nakano et al. who observed an effect of aluminum sulfate on nalidixic acid permeability through a cellulose membrane at acidic pH conditions [26]. On the contrary, toxicological studies have shown that the absorption of aluminum is enhanced if it is absorbed in the form of chelates with small organic acids like citric and lactic acid [27]. Furthermore, it was also reported that the transport of a heavy metal–fluoroquinolone complex into a bacterial cell is superior to the transport of the heavy metal or fluoroquinolone alone [28]. Unlike calcium, magnesium and iron, aluminum is not transported actively through the intestinal mucosa. Therefore it is also ideal for the evaluation of the influence of fluoroquinolones on the permeability of metal cations. These data were essential to test the hypothesis of "the formation of coordination compounds between fluoroquinolones and metal cations with very low or zero permeability" [12,2,13] and to finally understand whether the metal cation–fluoroquinolone complexes can permeate through the intestinal mucosa.

The amount of Al^{3+}, which was accumulated in the 2.5 ml acceptor compartment on the serosal side of the diffusion chambers in the 100 min long permeation through 1 cm^2 of rat jejunum, was measured in different conditions. When the donor (mucosal) compartment was "Al^{3+}-free", the measured amount of Al^{3+} in the 2.5 ml acceptor compartment was 0.6 ± 0.1 nmol (trace levels of aluminum are always present in biological and non-biological samples). In the second phase of the experiment, when the donor compartment was filled with 5 mM aluminum chloride in Ringer buffer, the amount measured on the other (serosal) side of the rat jejunum was only 1.0 ± 0.3 nmol (still at "trace level"). Finally, when ciprofloxacin and aluminum were both present in the donor compartment at 5 mM total concentrations, the amount of aluminum measured in the acceptor compartment was 0.7 ± 0.2 nmol, which is almost the same as in the case of the "Al^{3+} free" donor compartment. Furthermore, the measured amounts of Al^{3+} in the acceptor compartment are extremely low compared to the amount of ciprofloxacin ($n = 43 \pm 5$ nmol), which permeates through the 1 cm^2 of rat intestinal mucosa in 100 min in the presence of Al^{3+}. These permeability results for Al^{3+} through the rat intestine thus clarify the mechanism of fluoroquinolone permeability reduction in the presence of metal cations. They also confirm that the small intestinal mucosa is a very tight barrier for Al^{3+} ions. Because Al^{3+} is not carried through the intestinal mucosa in the presence of abundant amount of ciprofloxacin in the donor compartment either, one can conclude, that at physiologically and therapeutically relevant conditions tested in our study ciprofloxacin–Al^{3+} complexes do not permeate through the intestinal mucosa at all.

We thus experimentally confirmed the assumption that the complexes of fluoroquinolones and metal cations are probably too large, rigid and/or too polar to permeate through the intestinal mucosa [21,13].

4. Conclusions

In this work we showed that the interactions between the fluoroquinolones and the metal cations in the gastrointestinal tract result in lower fluoroquinolone bioavailability due to the decreased intestinal permeability of fluoroquinolones. It was also confirmed that the fluoroquinolone–metal cation complexes do not permeate through the intestinal mucosal membrane at all. This

was achieved with a novel approach in the analysis of drug permeability and by an application of a permeability–bioavailability correlation, which is normally used for the biopharmaceutical evaluation of drug candidates in the research of drug–drug interactions. Additional lowering of the fluoroquinolone bioavailability could be attributed to the integration of fluoroquinolones in/on the precipitates of metal cation hydroxides formed during the neutralization of gastric content with bicarbonate in the duodenum.

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