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Physicochemical properties of the fluoroquinolone antimicrobials V. Effect of fluoroquinolone structure and pH on the complexation of various fluoroquinolones with magnesium and calcium ions

Danna L. Ross [1] and Christopher M. Riley

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045 (USA)

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Summary

The complexation of nalidixic acid and nine 7-piperazinylfluoroquinolone antimicrobials with magnesium and calcium ions was studied at room temperature (22 ± 1°C) by spectrofluorometry. Scatchard plots were used to determined the stoichiometry of binding and the effects of pH were investigated to determine which Bjerrum species of the compounds bound most tightly to the metal ions. Multiple regression analysis was used to determine the relationship between the association constants and the binding to magnesium ions. The results of that analysis were then used to propose a structure for the 2:2 complex of magnesium and lomefloxacin.

Introduction

Complexation with metal ions commonly found in antacids and vitamin preparations is known to reduce the oral bioavailability of fluoroquinolone antimicrobials (Flor et al., 1985, 1990; Hoffken et al., 1985a,b, 1988; Frank et al., 1986; Lener et al., 1987; Schentag et al., 1988; Frost et al., 1989a,b; Grasela et al;., 1989; Nix et al., 1989a,b; Polk, 1989; Polk et al., 1989; Brouwers et al., 1990) as well as interfering with their antimicrobial activity in urine (Barbhaiya et al., 1982; Ratcliffe et al., 1983; Pohlod et al., 1984; Kumada et al., 1985; Smith et al., 1985, 1988; Blaser et al., 1988; Gurdal et al., 1990; Perez-Giraldo et al., 1990). In a recent paper, Ross and Riley (1992b) have demonstrated the relationship between the nature of the metal ion and the stability constants of the complexes formed between lomefloxacin

Correspondence to: C.M. Riley, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.

[1] *Present address:* 3M Pharmaceuticals, 3M Center, St. Paul, MN 55144, U.S.A.

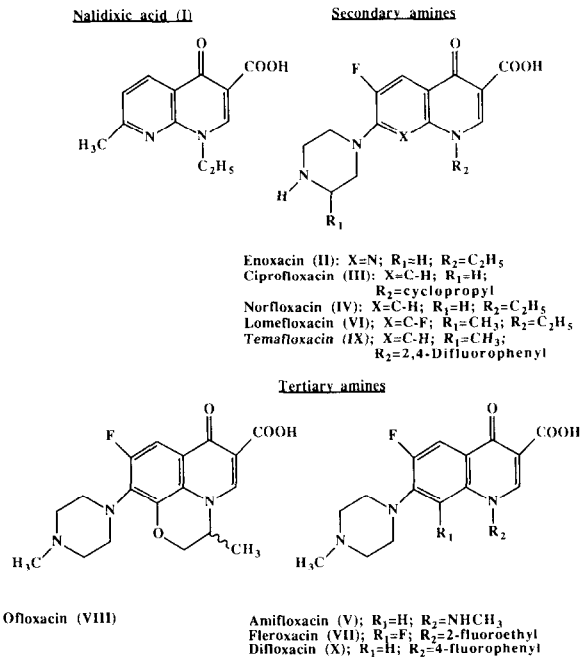

Fig. 1. Structures of the quinolones studied.

and Ca^{2+}, Mg^{2+}, Fe^{3+}, Bi^{3+} and Al^{3+}. The present study was concerned with the elucidating the effects of quinolone structure on the complexation with two representative metal ions, Mg^{2+} and Ca^{2+}. Because Ca^{2+} had been reported to affect bioavailability only at elevated pH values (Frost et al., 1989a,b) and because the activity of various quinolones in the presence of Mg^{2+} varied with pH in vitro (Smith and Ratcliffe, 1985; Perez-Giraldo et al., 1990) it was of interest to study the effect of pH on the formation of drug-metal ion complexes with both Ca^{2+} and Mg^{2+}. Therefore, the complexation of Ca^{2+} with lomefloxacin and the complexation of Mg^{2+} with several quinolones (Fig. 1) was studied as a function of pH.

In the previous investigation (Ross and Riley, 1992b), solubility was found to be a convenient method to study the complexation of all the metals ions of interest with lomefloxacin. In the present investigation, fluorescence was found to be the most convenient analytical method because the addition of Ca^{2+} or Mg^{2+} to lomefloxacin solutions resulted in a substantial increase in fluorescence intensity.

Theory

The fluorescence (F) of a solution containing the drug and a metal ion is the sum of the individual fluorescent species:

$$F = 2.3 I_0 l(\phi_S \epsilon_S [S] + \phi_{SL} \epsilon_{SL} [SL]) \quad (1)$$

where I_0 is the intensity of the exciting light, l denotes the optical pathlength, ϕ_S and ϕ_{SL} are the relative fluorescence quantum yields, and ϵ_S and ϵ_{SL} represent the molar absorptivities of the relevant species. The ligand ions (Ca^{2+} or Mg^{2+}) were not fluorescent and their concentrations were not included in eqn 1. In the absence of ligand, but in the presence of the same total concentration of the substrate (drug), the fluorescence intensity is:

$$F_0 = K_s S_t \quad (2)$$

For simplicity, here and later in the text, the ($2.3 I_0 l \phi_S \epsilon_S$) term has been combined into a proportionality constant, K, followed by the subscript describing the species involved (S for substrate, L for ligand and SL for the complex). Combining and rearranging Eqns 1 and 2 results in Eqn 3 that relates the change in fluorescence intensity and the ligand concentration to the binding constant, K_{11}:

$$\frac{F}{F_0} = \frac{1 + (K_{SL}/K_S) K_{11}[L]}{1 + K_{11}[L]} \quad (3)$$

The assumption that $[L] = [L]_t$ was been made for all fluorescence calculations because $[L] \gg [S]$. Eqn 3 may be transformed into its linear Scatchard form for the purpose of plotting the data:

$$\frac{(F/F_0) - 1}{[L]} = \frac{K_{SL}}{K_S} K_{11} - K_{11} \frac{F}{F_0} \quad (4)$$

Materials and Methods

Materials

All the quinolones were supplied by their respective manufacturers: amifloxacin (Sterling-Winthrop, Rensselaer, NY), ciprofloxacin HCl (Miles Laboratories, West Haven, CT), difloxacin HCl (Abbott Laboratories, North Chicago, IL), enoxacin (Warner-Lambert Co., Ann Arbor, MI), fleroxacin (Hoffmann-LaRoche Inc., Nutley, NJ), lomefloxacin mesylate and lomefloxacin HCl (G.D. Searle and Co., Skokie, IL), ofloxacin (Ortho Laboratories, Raritan, NJ), norfloxacin (Merck, Sharp, and Dohme, West Point, PA), and temafloxacin HCl (Abbott Laboratories). Nalidixic acid was purchased from Sigma Chemical Co (St. Louis, MO). Tris was gold Label (99.9 + %), purchased from Aldrich Chemical Co (Milwaukee, WI). All solvents were HPLC grade and obtained from commercial sources. All buffer components, magnesium chloride and calcium chloride were ACS reagent grade or better and obtained from commercial sources. All other chemicals were reagent grade obtained from commercial sources. Water was purified in a Milli-Q Water System (Millipore Corp., Bedford, MA) and stored in glass containers until use. Glassware was washed with nitric acid and rinsed with metal-free water before use to eliminate any trace metal contaminants and all buffers were checked for the presence of heavy metal ions using the dithizone test (Stout and Arnon, 1939).

Apparatus

All pH measurements were made using an Orion SA 520 pH meter (Orion Research, Inc., Boston, MA) and a Tiny Combination pH electrode (Microelectrodes, Inc., Londonderry, NH) or a calomel pH combination glass electrode (Markson, Phoenix, AZ). Fluorescence measurements were made using an SLM Aminco 4800 Spectrofluorometer (SLM Instruments, Inc., Urbana, IL) with a rhodamine B reference standard.

Complexation studies

The binding of calcium and magnesium ions to lomefloxacin was studied fluorophotometrically at ambient temperature (22 ± 1°C). Fluorescence intensity measurements were made at excitation and emission wavelengths of 282 and 420 nm, respectively. Linearity of fluorescence with respect to concentration, up to the concentrations used in these studies, was established at each pH value in the relevant buffer (acetate pH 5, 0.15 M; Tris pH 7 and 9, 0.1 M: $\mu = 0.35$ with NaCl). A series of solutions was prepared with a constant drug concentration and increasing metal-ion concentrations. The change in fluorescence with metal-ion concentration was then monitored. At each pH value, two drug concentrations which varied by at least 50% were studied. All fluorophotometric measurements were made at least in triplicate.

Results and Discussion

Scatchard plots

The Scatchard plots obtained by analysis of the data according to equation 4 were linear in all cases indicating formation 1:1 complexes of the fluoroquinolones studied (Fig. 1) and calcium or magnesium. A representative Scatchard plot is shown in Fig. 2. These results, along with the fact that no significant change in K_{11} resulted when the substrate concentration was increased by 50%, indicated that no higher order complexes were present. Although previous FAB-MS analysis (Ross and Riley, 1992b) had indicated the presence of 2:1 (drug:Mg^{2+}) complexes, the ratio of drug:Mg^{2+} in the MS studies was only 4:1. In

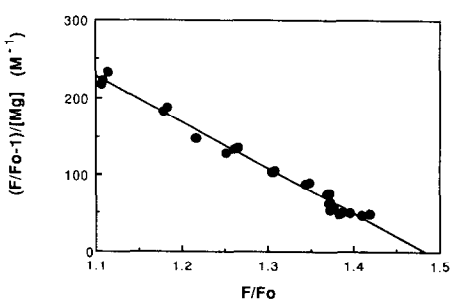

Fig. 2. Scatchard plot of temafloxacin HCl (8.09×10^{-6} M) and Mg^{2+} at pH 9 ($\mu = 0.35$). The value of the binding constant determined from the equation of the line was 602 M^{-1}.

the fluorescence studies, the Mg^{2+} concentrations were 1000-times higher than the drug concentrations and therefore the probability of 2:1 (drug:metal) complexes being formed was expected to be very low. Furthermore, Miller (1989) has indicated that stoichiometries of metal-ion complexes determined by FAB-MS should be treated with caution because of artifacts and reactions occurring in the radiation-damaged matrix. Using an MINSQ curve-fitting algorithm, the experimental data were fitted to Eqn 3. A representative plot of experimental data and theoretical curve shown in Fig. 3. The value for r^2 was greater than 0.999 in all cases.

Effects of pH on complexation

The complexation of an ionizable drug with a metal ion may vary with pH because each Bjerrum species would have its own unique stability constant with a given metal. Therefore, the observed binding constant at any pH would simply be the sum of the individual contributions:

$$K_{11} = K_{11_{H_2Q^+}} f_{H_2Q^+} + K_{11_{HQ^\pm}} f_{HQ^\pm} + K_{11_Q} f_{Q^-} \quad (5)$$

Furthermore, pH-dependent metal ion binding is particularly likely if the binding site is also the site of protonation because the metal will compete with the proton. As expected, the binding constants for the fluoroquinolones were found to vary significantly as a function of pH (Tables 1 and 2). The results of these studies indicate that for Ca^{2+} and Mg^{2+}, the affinity of the metal for the anion > zwitterion ≫ cation. In the case of Ca^{2+}, the cation did not bind the metal. These results are consistent with the clinical studies of Frost et al. (1989b) which suggested that complexation of ciprofloxacin with calcium occurred only at elevated pH values.

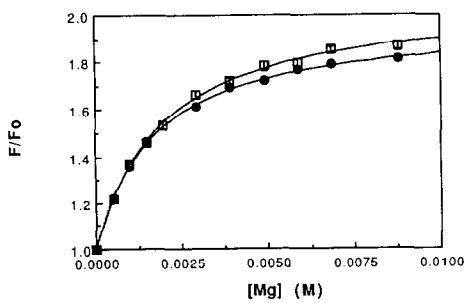

Fig. 3. Change of fluorescence as a function of Mg^{2+} concentration for temafloxacin HCl: 8.09×10^{-6} M (●) and 4.05×10^{-6} M (□) at pH 9 (μ = 0.35 with NaCl). Calculated curves generated using Eqn 3 and binding constants from Table 1.

TABLE 1

The binding constants of Ca^{2+} with lomefloxacin studied as a function of pH and drug concentration determined by fluorescence

pH	Drug concentration (M) ($\times 10^6$)	$K_{11} \pm$S.D. (M^{-1})
4.96	4.01	5± 1
4.99	2.01	17± 4
7.04	7.77	120± 9
7.06	3.88	43± 6
7.15	4.00	43± 7
9.18	9.19	79±11
9.17	4.01	156±17

The binding constants at each pH value studied for each of the drugs with Mg^{2+} are reported in Table 2. Cole et al. (1984) reported the binding constant of nalidixic acid and Mg^{2+} determined potentiometrically to be 1122 at 37°C at μ = 0.15. Timmers and Sternglanz (1978) used spectrophotometric methods at pH 7.5 to determine a binding constant of 1000 between nalidixic acid and Mg^{2+} and a binding constant of 158 between nalidixic acid and Ca^{2+}. Both these groups reported finding only 1:1 binding between nalidixic acid and these divalent cations. Although the stoichiometry they found is consistent with the results of the studies reported here, the binding constants reported by both groups was higher than the values reported in the present study (pH 7, K_{11} (Mg^{2+}-nalidixic acid) = 606, K_{11} (Ca^{2+}-lomefloxacin) = 63). Behrens and Mendoza Diaz (1986) reported the formation of 2:1 (drug:metal) complexes between nalidixic acid and both Mg^{2+} and Ca^{2+}, again finding higher order solid complexes than observed in our fluorescence studies as the predominant species in solution. The presence of 2:1 (drug:metal) complexes was indicated for Mg^{2+}-lomefloxacin by mass spectroscopy at a drug:metal ratio of 4:1 (Ross and Riley, 1992b) and therefore, the absence of evidence of 2:1 (drug:metal) complexes in the fluo-

rescence studies may be due to the excess of metal ion present.

To determine if differences in the structures of the quinolones had any effect on the binding constants, the complexation of Mg^{2+} with nine fluoroquinolones was studied. Because binding of Mg^{2+} to the quinolones varied with pH, the effect of pH on the binding constant was also studied for each quinolone-Mg^{2+} complex (Table 2).

In all cases, the binding of the drug to Mg^{2+} increased with pH. As with Ca^{2+}, no binding to the cationic species occurred with any of the drugs studied. The failure of the cationic species to bind Mg^{2+} may be indicative of involvement of the carboxylic acid moiety in the coordination site with the affinity of the metal ion being insufficient to displace the carboxylic acid proton; however, the failure of the cationic species to bind with Mg^{2+} could also involve repulsive forces between the positively charged drug molecule and Mg^{2+}. Once the carboxylic acid proton was dissociated, binding to Mg^{2+} occurred with the binding constant associated with the anion being larger in all cases than that associated with the zwitterionic or neutral species. A simple explana-

TABLE 2

Binding constants of Mg^{2+} with the quinolone compounds as a function of pH

Compound	pH	Concentration (μM)	$K_{11} \pm$ S.D. (M^{-1})	Compound	pH	Concentration (μM)	$K_{11} \pm$ S.D. (M^{-1})
Amifloxacin	6.14	8.13	418 ± 15				
	6.13	4.08	348 ± 21				
	6.96	8.29	672 ± 11	Lomefloxacin	5.01	3.80	71 ± 11
	6.96	4.15	641 ± 11		5.03	1.52	82 ± 11
	8.93	8.49	718 ± 25		6.91	7.92	470 ± 42
	8.94	4.25	748 ± 23		6.93	3.96	395 ± 49
	8.97	9.23	743 ± 21		8.89	7.79	615 ± 61
	8.97	4.62	727 ± 18		8.91	3.90	496 ± 60
Ciprofloxacin	5.00	3.90	50 ± 6	Nalidixic	5.33	8.09	51 ± 12
	5.00	1.56	50 ± 4	acid	5.34	4.05	114 ± 12
	6.92	7.93	710 ± 30		6.89	8.91	605 ± 16
	6.93	3.97	672 ± 14		6.91	4.46	607 ± 26
	8.94	8.22	888 ± 16		8.92	9.42	556 ± 9
	8.95	4.11	918 ± 22		8.93	4.71	599 ± 24
Difloxacin	5.04	3.96	46 ± 6	Norfloxacin	5.02	3.87	4 ± 6
	5.03	1.58	63 ± 6		5.01	1.55	22 ± 6
	6.93	7.95	519 ± 11		6.88	8.12	751 ± 16
	6.94	3.98	469 ± 16		6.89	4.06	689 ± 23
	8.95	7.97	726 ± 26		8.95	8.24	1 115 ± 40
	8.97	3.99	610 ± 22		8.95	4.12	1 177 ± 66
Enoxacin	5.01	3.20	39 ± 10	Ofloxacin	5.02	6.00	53 ± 2
	5.01	1.60	53 ± 15		5.02	3.00	54 ± 3
	6.93	8.29	650 ± 22		6.91	7.53	416 ± 47
	6.93	4.15	693 ± 28		6.92	3.77	567 ± 79
	8.94	8.50	1 008 ± 49		8.95	7.74	676 ± 18
	8.94	4.25	988 ± 66		8.95	3.87	686 ± 21
Fleroxacin	5.32	6.13	134 ± 0	Temafloxacin	5.33	6.20	118 ± 2
	5.34	3.07	159 ± 4		5.34	3.10	123 ± 2
	6.90	7.92	465 ± 11		6.95	7.82	412 ± 10
	6.90	3.65	357 ± 21		6.96	3.91	402 ± 11
	8.94	8.28	600 ± 20		8.96	8.09	605 ± 13
	8.94	4.14	623 ± 15		8.97	4.05	532 ± 13

tion for this phenomenon was that as the pH increased, the binding constant continued to increase because of a decrease in electrostatic repulsion, i.e., the positive charges on the zwitterions made it harder for the Mg^{2+} to approach the binding site. When the anion is the predominant species, the maximal binding could occur because there was no longer any protonated carboxylate species and there was no electrostatic repulsion due to the protonated piperazinyl species. Another possible explanation for the increased binding of the anionic species was that the piperazinyl nitrogen was participating in the coordination with the metal ion.

Relationship between structure and complexation

To understand further the differences in the binding constants resulting from changes in the structure of the various drugs, multiple-regression analysis was performed looking at several structural features which varied between the different drugs. The structural features which were found to affect the K_{11} value included the number of fluorines, the presence or absence of a 4′-*N*-methyl piperazinyl substituent, and the presence or absence of a 3′-methyl substituent on the piperazinyl ring. Because the number of fluorines did not account for differences in the electron-withdrawing effect due to the distance between the fluorine atom and the carboxylic acid site (Ross and Riley, 1992a), the pK_{a_1} was used in the multiple regression as an indicator of the electron density of the carboxylic acid moiety. The general regression equation resulting from analysis correlating the effects of the pK_{a_1}, the presence (1) or absence (0) of a 4′-*N*-methyl substituent (m), the presence (1) or absence (0) of a 3′-methyl piperazinyl function (3′m), and the pH with the log K_{11} value was:

$$\log K_{11} = 1.5(\pm 0.20) + 0.068(\pm 0.007)\text{pH} + 0.14(\pm 0.031)pK_{a_1} - 0.17(\pm 0.025)3'\text{m} - 0.12(\pm 0.017)\text{m} \quad (6)$$

$$R^2 = 0.90,\ p = 0.0001$$

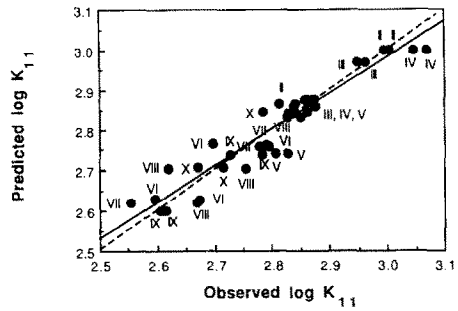

Fig. 4. Results of a multiple regression analysis for K_{11} values for Mg^{2+} (Table 2) and the various fluoroquinolones. Compounds are identified by the numbers corresponding to the structures in Fig. 1. The predicted log K_{11} was calculated from the regression equation determined to be: log $K_{11} = 1.5 + 0.68$ pH $+ 0.14$ $pK_{a_1} - 0.17$ (absence (0) or presence (1) of a 3′-methyl substituent) $- 0.12$ (absence (0) or presence (1) of a 4′-*N*-methyl substituent). The regression line is shown by the solid line and fits the equation: $y = 0.28 + 0.90x$. The coefficient of correlation (R) is 0.95. The dashed line represents the theoretical line of unit slope and zero intercept.

Fig. 4 is a graphical representation of the regression results (Eqn 6). Nalidixic acid was not included in this evaluation because it did not contain a piperazinyl function.

The regression model (Eqn 6) demonstrated that an increase in the pK_{a_1} value resulted in an increase in the binding constant. The change in the dissociation constant was an indicator of the electron density near the protonation site. As the electron density near the protonation site increased, the anionic form was less stable than the protonated form which resulted in an increase in the pK_1 value. An increase in electron density would be expected to increase the attraction of the metal ion for the drug molecule resulting in an increased binding constant.

The changes in the K_{11} values seen in those compounds containing the 3′-methyl or 4′-*N*-methyl substituents could only be explained if the 4′-piperazinyl nitrogen participated in the complexation. If a 4′-*N*-methyl substituent was present, the amine was tertiary and the resulting K_{11} values may be lower than with secondary amines because of steric hindrance from the methyl substituent resulting in less interaction between the nitrogen and metal ion. The presence of a 3′-methyl substituent again may interfere with the

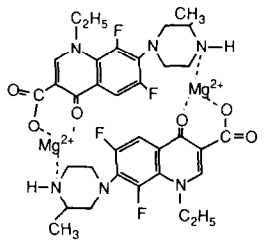

Fig. 5. Proposed structure of a 2:2 complex between Mg^{2+} and lomefloxacin.

interaction between the nitrogen and metal ion due to steric hindrance; therefore, the presence of a 3′-methyl substituent resulted in a decrease in the binding constant. The fact that the pK_{a_1} and the presence of the 3′-methyl and 4′-*N*-methyl substituent both affect the binding constant suggests that both the carboxylic acid moiety and the piperazinyl nitrogen may participate in the complexation with magnesium. In order for this to occur, adjacent molecules would have to interact resulting in a 2:2 complex. Because the presence of a 3′-methyl or a 4′-*N*-methyl results in only a small decrease in the binding constant, it is likely that the piperazinyl nitrogen serves to stabilize the complex but could not be considered the primary binding site.

Possible structure of the magnesium-lomefloxacin complex

Fig. 5 shows the proposed structure of the 2:2 complex between lomefloxacin and the magnesium ion. The proposed 2:2 complex is quite consistent with the Scatchard plots (e.g., Fig. 2) which simply indicates that the ratio of drug:metal in the complex was 1:1, 2:2, etc. For a given ligand, the geometry of the complex, as well as the stability and the type of atom involved (O, N, etc.) in the coordination sphere of the complex may vary with the metal ion. For example, complexes with Mg^{2+} and Ca^{2+} form primarily with oxygen ligands (Cotton et al., 1980). Although complexation with nitrogen ligands occurs in the solid state, it is fairly weak and generally dissociation of the complex occurs in aqueous solution. However, Mg^{2+} complexes with tetrapyrrole systems, such as the chlorophylls, forming a five-coordinate square pyramidal complex. Both Ca^{2+} and Mg^{2+} form stable five-coordinate complexes with oxygen ligands as well. Complex formation between EDTA and Ca^{2+} occurs in alkaline solution and results in formation of a 1:1 complex. The antibiotic, A23187, which is a monocarboxylic acid, is a tridentate (N, O, O) ligand and forms a seven-coordinate Ca^{2+} complex (Cotton and Wilkinson, 1980).

Although participation of the aromatic ring nitrogen (1-N) cannot be discounted with the quinolones, the 3,4 β-diketone moiety appears to be the most likely primary site of complexation with Ca^{2+} and Mg^{2+}. The fact that neither of these metals bind to the cationic form of the drug and that binding increased with increasing electron density near the carboxylic acid function was a good indication that the carboxylic acid site was involved and that the affinity of the metal was not strong enough to displace the carboxylic acid proton. Since the carboxylic acid proton was hydrogen bonded to the 4-keto functionality, it was reasonable to postulate that the 4-keto moiety may also be involved in the binding of drug to the metal ions. Further, in a study conducted by Bailey et al. (1984), the Mg^{2+} binding of two analogs of nalidixic acid, i.e., 1-ethyl-1,4-dihydro-7-methoxy-4-oxoquinoline-3-carboxylic acid (MQC) and its corresponding homologous acid, 2-(1-ethyl-1,4-dihydro-7-methoxy-4-oxoquinol-3-yl)ethanoic acid (MQE), was determined. Using a computer simulation, the distribution of MQE and MQC in plasma was modeled. The Mg^{2+}-MQC complex involved 65.3% of the total MQC in plasma while the Mg^{2+}-MQE complex only involved 14.1% of the total MQE in plasma. The differences in binding was consistent with involvement of the 3,4 β-diketone in the coordination site. The introduction of a methylene group between the aromatic ring and the carboxylic acid in MQE resulted in a decreased binding which can be attributed to the relative instability of a seven-membered chelate ring as compared to the six-membered chelate ring formed with the MQC compound (Bailey et al., 1984).

Conclusions

Linear Scatchard plots indicated that the stoichiometry of complexation of the fluoroquinolones with calcium and magnesium was 1 : 1. The effects of pH on the complexation indicated that the affinity of magnesium or calcium for the anion > zwitterion > cation, suggesting that the primary binding site was the carboxylic acid. This hypothesis was supported by multiple regression analysis, which showed that the association constant was related to the ionization constant, and thus the electron density, of the carboxylic acid moiety. However, the regression analysis indicated that the association constant was also related to the presence of C3′- and N4′-methyl groups on the piperazine ring, implicating the 4′-nitrogen in the complexation. Based on these results a 2:2 stoichiometry for the magnesium (and calcium) complexes of the 7-piperazinylfluoroquinolones was proposed involving coordination at the carboxylic acid, the adjacent 4-keto group and the 4′-nitrogen of the piperazine ring.

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References

Bailey, A., Cole, A., Goodfield, J., May, P.M., Dreyfuss, M.E., Midgley, J.M. and Williams, D.R., The complexation of transition series metal ions by nalidixic acid and related methoxyquinolones: its influence on partition coefficients with reference to antibacterial activity. *Int. J. Pharm.*, 22 (1984) 283–290.

Barbhaiya, R., Gerber, A., Craig, W. and Welling, P., Influence of urinary pH on the pharmacokinetics of cinoxacin in humans and on antibacterial activity in vitro. *Antimicrob. Agents. Chemother.*, 21 (3) (1982) 472–480.

Behrens, N. and Mendoza-Diaz, G., Metal complexes of the antibiotic nalidixic acid. *Inorg. Chim. Acta*, 125 (1986) 21–26.

Blaser, J. and Luthy, R., Comparative study on antagonistic effects of low pH and cation supplementation on in-vitro activity of quinolones and aminoglycosides against Pseudomonas aeruginosa. *J. Antimicrob. Chemother.*, 22 (1988) 15–22.

Brouwers, J., Van der Kam, H., Sijtsma, J. and Proost, J., Decreased ciprofloxacin absorption with concomitant administration of ferrous fumarate. *Pharm. Weekbl. Sci. Ed.*, 12 (1990) 182–183.

Cole, A., Goodfield, J., Williams, D.R. and Midgley, J.M., The complexation of transition series metal ions by nalidixic acid. *Inorg. Chim. Acta*, 92 (1984) 91–97.

Cotton, A. and Wilkinson, G., *Advanced Inorganic Chemistry*, 4th Edn, Wiley, New York, 1980, pp. 283–284.

Flor, S., Guay, D., Opsahl, J., Tack, K. and Matzke, G., Effects of magnesium-aluminum hydroxide and calcium carbonate antacids on bioavailability of ofloxacin. *Antimicrob. Agents Chemother.*, 34 (1990) 2436–2438.

Flor, S., Weintraub, H., Marriott, T., Freidmann, N. and Beals, B., Pharmacokinetics of ofloxacin in humans after various single oral doses. In Ishigami, J. (Ed.), *Recent Advances in Chemotherapy, Antimicrobial Sect. 2, Proc. 14th Int. Congr. Chemother., Kyoto*, University of Tokyo Press, Tokyo, 1985, pp. 1783–1784.

Frank, W., Peace, K., Watson, J., Szego, P., Braverman, A., Mico, B. and Dickson, B., The effect of single intravenous doses of cimetidine or ranitidine on gastric secretion. *Clin. Pharmacol. Ther.*, 40 (1986) 665–672.

Frost, R., Carlson, J., Dietz, A., Heyd, A. and Lettieri, J., Ciprofloxacin pharmacokinetics after a standard or high-fat/high-calcium breakfast. *J. Clin. Pharmacol.*, 29 (1989a) 953–955.

Frost, R., Lettieri, J., Noe, A., Shamblen, E. and Lasseter, K., Effect of aluminum hydroxide and calcium carbonate antacids on ciprofloxacin bioavailability. *Clin. Pharmacol. Ther.*, 45 (1989b) 165.

Grasela Jr., T., Schentag, J., Sedman, A., Wilton, J., Thomas, D., Shultz, R., Lebsack, M. and Kinkel, A., Inhibition of enoxacin absorption by antacids or ranitidine. *Antimicrob. Agents Chemother.*, 33 (1989) 615–617.

Gurdal, H., Tulunay, F. and Altay, G., Post antibiotic effect of ofloxacin and the activity of Mg^{++}. *J. Antimicrob. Chemother.*, 26 (1990) 291–292.

Hoffken, G., Lode, H., Prinzing, C., Borner, K. and Koeppe, P., Pharmacokinetics of ciprofloxacin after oral and parenteral administration. *Antimicrob. Agents Chemother.*, 27 (1985a) 375–379.

Hoffken, G., Lode, H., Wiley, R., Glatzel, P., Borner, K. and Koeppe, P., Interactions on the gastrointestinal absorption of ciprofloxacin. In Ishigami, J. (Ed.), *Recent Advances in Chemotherapy, Antimicrobial, Sect. 2, Kyoto, Proc. 14th Int. Congr. Chemother.*, Kyoto, University of Tokyo Press, Tokyo, 1985b, pp. 1606–1607.

Hoffken, G., Lode, H., Wiley, R., Glatzel, T., Sievers, D., Olschewski, T., Borner, K. and Koeppe, T., Pharmacoki-

netics and bioavailability of ciprofloxacin and ofloxacin: effect of food and antacid intake. *Rev. Infect. Dis.*, 10 (Suppl. 1) (1988) S138–S139.

Kumada, T., Ooi, S., Totsuka, K. and Shimizu, K. (1985). Antimicrobial activity of quinolone antibiotics in urine. In Ishigami, J. (Ed.), *Recent Advances in Chemotherapy, Antimicrobial, Sect. 2, Proc. 14th Int. Congr. Chemother., Kyoto*, University of Tokyo Press, Tokyo, 1985, pp. 1881–1882.

Lener, M., Watson, A., Krol, G., Goldstein, H., Frost, W., Lettieri, J. and Schentag, J., Antacid inhibition of ciprofloxacin in normal volunteers. *Pharm. Res.*, 4 (1987) S-79.

Miller, J., Fast atom bombardment mass spectrometry (FAB MS) of organometallic, coordination, and related compounds. *Mass Spectrosc. Rev.*, 9 (1989) 319–347.

Nix, D., Watson, W., Lener, M., Frost, R., Krol, G., Goldstein, H., Lettieri, J. and Schentag, J., Effects of aluminum and magnesium antacids and ranitidine on the absorption of ciprofloxacin. *Clin. Pharmacol. Ther.*, 46 (1989a) 700–705.

Nix, D., Wilton, J., Schentag, J., Parpia, S., Norman, A. and Goldstein, H., Inhibition of norfloxacin absorption by antacids and sucralfate. *Rev. Infect. Dis.*, 11 (Suppl. 5) (1989b) S1096.

Perez-Giraldo, C., Hurtado, C., Moran, F. and Blanco, M., The influence of magnesium on ofloxacin activity against different growth phases of *Escherichia coli. J. Antimicrob. Chemother.*, 25 (1990) 1021–1026.

Pohlod, D.J. and Saravolatz, L.D., In vitro susceptibilities of 393 recent clinical isolates to WIN 49375, cefotaxime, tobramycin, and piperacillin. *Antimicrob. Agents Chemother.*, 25 (1984) 377–379.

Polk, R., Drug-drug interactions with ciprofloxacin and other fluoroquinolones. *Am. J. Med.*, 87 (Suppl. 5A) (1989) 76S–81S.

Polk, R., Healy, D., Sahai, J., Drwal, L. and Racht, E., Effect of ferrous sulfate and multivitamins with zinc on absorption of ciprofloxacin in normal volunteers. *Antimicrob. Agents Chemother.*, 33 (1989) 1841–1844.

Ratcliffe, N. and Smith, J., Effects of magnesium on the activity of 4-quinolone antimicrobial agents. *J. Pharm. Pharmacol.*, 35 (1983) 61P.

Ross, D.L. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. II: Acid ionization constants and their relationship to structure. *Int. J. Pharm.*, 83 (1992a) 267–272.

Ross, D.L. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. III: Complexation of lomefloxacin with various metal ions and the effect of metal ion complexation on aqueous solubility. *Int. J. Pharm.*, 87 (1992b) 203–213.

Schentag, J., Watson, W., Nix, D., Sedman, A., Frost, R. and Letteri, J., Time dependent interactions between antacids and quinolone antibiotics. *Clin. Pharmacol. Ther.*, 43 (1988) 135.

Smith, J. and Lewin, C., Chemistry and mechanisms of action of the quinolone antibacterials In Andriole, V. (Ed.), *The Quinolones*, Academic Press, San Diego 1988, pp. 23–82.

Smith, J. and Ratcliffe, N., Effect of pH and magnesium on the in vitro activity of ciprofloxacin. In Neu, H. and Weuta, H. (Eds.), *First International Ciprofloxacin Workshop*, Excerpta Medica, Leverkusen, 1985.

Stout, P. and Arnon, D., Experimental methods for the study of the role of copper, managanese, and zinc in the nutrition of higher plants. *Am. J. Bot.*, 26 (1939) 144–149.

Timmers, K. and Sternglanz, R., Ionization and divalent cation dissociation constants of nalidixic and oxolinic acids. *Bioinorg. Chem.*, 9 (1978) 145–155.