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Review

Effect of metal ions on some pharmacologically relevant interactions involving fluoroquinolone antibiotics

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Abstract

Background: Complexation of five metal cations, Fe^{3+}, Al^{3+}, Zn^{2+}, Cu^{2+} and Mg^{2+} with four fluoroquinolones, levofloxacin, sparfloxacin, ciprofloxacin hydrochloride and enrofloxacin and human serum albumin (HSA) has been studied for better understanding of bioavailability of drugs interacting with metals and proteins.

Methods: The binding parameters have been determined using fluorescence and ultraviolet absorption spectroscopic techniques. The effect of metal cations on the interaction of fluoroquinolones with HSA has also been investigated.

Results: The association constants were of the order of 10^2–10^4 for the fluoroquinolone-metal ion interaction. For a given drug, the chelation potential of Al^{3+} was highest, whereas that of Mg^{2+} was lowest. At a metal ion/drug ratio of 1:1, approximately 50%–73% of metal ion was bound per mole drug in most cases. In the case of HSA-metal ion interaction, for Fe^{3+} and Zn^{2+} ions, there was only one class of binding site, whereas for Al^{3+} and Cu^{2+} ions, two types of binding sites were found. The relative affinity of various metal ions was found to vary as $Al^{3+}>Cu^{2+}>Zn^{2+}>Fe^{3+}$. The extent of binding was found to be independent of the charge on the ion. Owing to very weak quenching of fluorescence, the association constant for the interaction of Mg^{2+} ion could not be determined by this technique. The binding affinity of all the fluoroquinolones to HSA was found to increase in the presence of Cu^{2+} ions, whereas all other metal ions decreased the binding affinity with the exception of levofloxacin in the presence of Zn^{2+} and Al^{3+} ions. Increase in the binding affinity indicated that the metal ions facilitate HSA-fluoroquinolone interaction and fluoroquinolones probably interact with HSA via a metal ion bridge. Decrease in the binding affinity, by contrast, can either be due to the fact that fluoroquinolone-metal ion complex inhibits fluoroquinolone-HSA interaction or metal ions produce conformational changes in the HSA molecule.

Conclusions: Results indicate that metal chelate formation can cause significant reduction in the antimicrobial activity of fluoroquinolone antibiotics. Alteration in the HSA-fluoroquinolone binding affinity in the presence of metal ions could have significant pharmacological effects. Quantitative estimate of the magnitude of interaction of different metal ions could also be obtained from the data.

Keywords: albumin; fluoroquinolone; interactions; metal ion.

Introduction

Metal ions play an essential role in the biological system. Various categories of drugs such as quercetin (1), catechol derivatives (2), tetracyclines (3) are known to form drug-metal chelates. The formation of metal chelates is believed to be the primary reason for reduced activity of these drugs in the presence of metal ion-containing pharmaceutical preparations. All fluoroquinolones also interact with multivalent cations (4, 5) and clinical investigations have shown that oral absorption and hence the antimicrobial activity of fluoroquinolones is reduced in the presence of metal ions (6, 7). Complexation with metal ions also alters the solubility, lipophilicity and protein binding of quinolones (8). The proposed mechanism of interaction is the chelation between the metal cation and the 3-carboxylate and 4-carbonyl group of the fluoroquinolones (5). Fluoroquinolone-metal ion interactions have been studied by some researchers (7, 8). However, detailed studies using different spectroscopic techniques are not available.

Serum albumin also associates with positively charged inorganic ions at physiologically relevant metal ion binding sites in a reversible manner and thus plays a major role in the transport of metal ions in blood plasma (9, 10). Metal ion binding to serum albumin has been extensively reported (11, 12). However, in most of these studies, the bovine serum albumin and equilibrium dialysis technique has been used. Very few detailed studies have been reported for human serum albumin (HSA) using spectroscopic methods. There appears to be no report for the complexation of ferric ions with serum albumin.

Drug-albumin interactions are important because most of the administered drugs are extensively and reversibly bound to serum albumin and the drug is transported mainly as a complex with protein. Because metal ions can interact with drugs as well as protein, the presence of metal ions can also have a significant effect on drug-protein interactions. Such studies on antihypertensive and antifungal agents, efonidipine (13), calcium channel blocker, azelnidipine (14) and vitamin K_3 (15) have been reported. The increase/decrease in the

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binding constants for different systems has been interpreted in terms of prolonged storage time of drug in plasma, reduced/enhanced pharmacological effect and altered elimination rate of the drug. The effect of metal ions on the interaction between some fluoroquinolones and HSA has also been reported (16, 17). However, detailed studies on various aspects of the problem are not available.

In the present study, four fluoroquinolones [levofloxacin, sparfloxacin, ciprofloxacin hydrochloride (HCI) and enrofloxacin] and five metal cations (Fe^{3+}, Zn^{2+}, Cu^{2+}, Mg^{2+} and Al^{3+}) have been selected. Whereas Fe^{3+}, Zn^{2+}, Cu^{2+} and Mg^{2+} play important physiological roles, Al^{3+}, which enters the body as a pollutant, has been recognized as a neurotoxic agent. Three types of interactions, drug-metal ion, HSA-metal ion and drug-HSA, in the absence and presence of metal ions have been studied using spectroscopic techniques.

Materials and methods

Pure drug samples were obtained as a gift: sparfloxacin was obtained from Cadila Pharmaceuticals Ltd., Dholka, India; levofloxacin, ciprofloxacin HCl and enrofloxacin were obtained from Ranbaxy Laboratories Ltd., Toansa, Punjab, India. Human serum albumin (HSA) was purchased from Sigma Chemical Co., St. Louis, MO, USA. All other reagents were of analytical grade. Water used was double-distilled in all glass apparatus. HSA solutions were prepared based on molecular weight of 66,500 Da. All metal ions were used as their chlorides ($FeCl_3$, $CuCl_2{\cdot}2H_2O$, $ZnCl_2$, $MgCl_2{\cdot}6H_2O$, $AlCl_3$). Owing to low solubility of metal chlorides at physiological pH, 0.05 M glycine buffer (pH 6.4) was used for these studies. Because buffer components can also bind metal ions, all the measured association constants are apparent association constants. A Perkin Elmer fluorescence spectrophotometer (PerkinElmer Inc., Waltham, MA, USA) equipped with a 150 W xenon lamp source and a Jasco V-530 ultraviolet absorption spectrophotometer (JASCO Inc., Easton, USA) were used.

Drug-metal ion interaction

Fluorescence spectroscopic technique A fluorescence spectroscopic technique was used in the case of enrofloxacin, levofloxacin and ciprofloxacin HCl only because sparfloxacin has no intrinsic fluorescence. In this regard, 2 mL of 50 µM drug solution was taken in a quartz cell and increasing amounts of metal ion stock solution (4 mM in the case of Fe^{3+}, Cu^{2+}, Zn^{2+} and Al^{3+} and 75 mM in the case of Mg^{2+}) was added. The concentration of drug was kept fixed at 50 µM by adding the same volume of 100 µM drug solution to the cell and metal ion concentration was varied in the range of 0.05–0.50, 0.05–1.00, 0.05–0.70, 0.025–0.500 and 1.50–15.00 mM in the case of Cu^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+} and Mg^{2+}, respectively. Owing to the low binding of Mg^{2+} with fluoroquinolones, a much higher concentration of Mg^{2+} had to be used. The fluorescence spectra were recorded in the range of 360–520 nm, after excitation at appropriate wavelength (272, 273 and 288 nm in the case of enrofloxacin, ciprofloxacin HCl and levofloxacin, respectively). The intrinsic fluorescence of drug was monitored at 462, 440 and 480 nm, in the case of enrofloxacin, ciprofloxacin HCl and levofloxacin, respectively. Data were analyzed as follows.

On the basis of the reports in the literature (7, 18), drug to metal stoichiometry was assumed to be 1:1. The association constant (K) for the interaction between drug (D) and metal ions (M), $D + M \rightleftharpoons D - M$ can be written as:

$$K = \frac{[D-M]}{[D_f][M_f]} = \frac{[M_b]}{[D_f][M_f]} \quad [1]$$

where $[D-M]=[D_b]=[M_b]$ is the bound drug/metal ion concentration, $[M_f]$ and $[D_f]$ are free metal ion and drug concentrations, respectively. The amount bound is expressed in terms of the moles of metal ion bound per mole drug (r). If $[D_t]$ is the total drug concentration then

$$r = \frac{[M_b]}{[D_t]} = \frac{[M_b]}{[D_f]+[D_b]} = \frac{[M_b]}{[D_f]+[M_b]} \quad [2]$$

substitution of $[M_b]=K\,[D_f]\,[M_f]$ in Eq. [2] leads to the familiar expression for a single binding site,

$$r = \frac{K[M_f]}{1+K[M_f]} \quad [3]$$

Inversion of Eq. [3] gives

$$\frac{1}{r} = \frac{1}{K[M_f]} + 1 \quad [4]$$

where r and $[M_f]$ values were determined from fluorescence data in the same manner as described earlier (19, 20). Association constant (K) for the interaction was determined from the linear 1/r vs. $1/[M_f]$ plots.

Ultra-violet (UV) absorption spectroscopic technique In this regard, 2 mL of 50 µM drug solution was titrated with the five metal ions in the same way as described above for the fluorescence method. Again, drug concentration was kept fixed (50 µM) and metal ion concentration was in the range of 0.04–0.35 mM for Fe^{3+}, 0.02–0.15 mM for Al^{3+}, 0.04–1 mM for Zn^{2+}, 0.04–0.50 mM for Cu^{2+} and Mg^{2+}. As compared to the fluorescence technique, much higher drug concentrations had to be used to obtain a measurable change in absorbance. After each addition of metal ion, UV absorption spectra (200–500 nm) were measured against glycine buffer reference. The metal ions used had no absorption in the UV region. Data were analyzed at the λ_{max} of various drugs: 272, 273, 286 and 288 nm in the case of enrofloxacin, ciprofloxacin HCl, levofloxacin and sparfloxacin, respectively. The association constant K for drug-metal ion binding was calculated from the linear double reciprocal, $1/\Delta A$ vs. $1/D_t$ plots using the Benesi-Hildebrand equation (21):

$$\frac{1}{\Delta A} = \frac{1}{K\Delta\varepsilon[D_t]}\frac{1}{[M_t]} + \frac{1}{\Delta\varepsilon[D_t]} \quad [5]$$

where $\Delta A=A_0-A$, A_0 and A are the absorbance of drug in the absence and presence of metal ions. $\Delta\varepsilon(=\varepsilon_b-\varepsilon_f)$ is the difference in the molar absorbance of bound and free drug. $[D_t]$ and $[M_t]$ are the total drug and metal ion concentration, respectively.

HSA-metal ion interaction

Interaction of metal ions with HSA was also studied at 27°C by the fluorescence spectroscopic technique using the same procedure as described earlier for HSA-drug interaction (19, 20). HSA concentration was kept fixed at 10 µM and metal ion concentration was varied in the range of 0.2–1, 0.02–0.25, 0.04–0.40 and 0.60–25 mM in the case of Cu^{2+} and Zn^{2+}, Al^{3+}, Fe^{3+} and Mg^{2+}, respectively. Intrinsic fluorescence of HSA was monitored at 334 nm after excitation at 295 nm. The association constants for interaction were calculated using the double reciprocal method, described in the previous section.

Drug-HSA interaction in the presence of metal ions

The effect of five metal ions (Fe^{3+}, Cu^{2+}, Zn^{2+}, Mg^{2+} and Al^{3+}) on the binding of fluoroquinolones (sparfloxacin, ciprofloxacin HCl, levofloxacin and enrofloxacin) to HSA has been studied at 27°C in glycine buffer (pH 6.4) using the fluorescence spectroscopic technique. HSA+metal ion was titrated with the drug in each case. Again, HSA concentration was kept fixed at 10 µM and metal ion concentration was fixed at 1000 µM in all cases, except enrofloxacin in the presence of Al^{3+} ions where 200 µM metal ion concentration was used. Intrinsic fluorescence of HSA was monitored at 334 nm after excitation at 295 nm. The methodology used for calculation of binding parameters was the same as described earlier (19, 20). The association constants for fluoroquinolone-HSA interaction in the absence and presence of various metal ions were calculated from the Scatchard plots.

The association constant data has been reported as average of three measurements. In all cases, the standard error of the mean (±SEM), calculated using statistical software SPSS for Windows (SPSS Inc., Chicago, IL, USA), was $< \pm 0.050 \times 10^x$, where 10^x is the multiplication factor which varies with the type of interaction involved.

Results and discussion

The structures of studied fluoroquinolone antibiotics are shown in Figure 1.

Drug-metal ion interaction

Fluorescence technique Intrinsic fluorescence of the drug was monitored in the absence and presence of increasing concentrations of various metal ions. This method could not be used for sparfloxacin because this drug does not have any intrinsic fluorescence. In most cases, the interaction was accompanied by a shift in fluorescence emission wavelength of the drug, indicating formation of a drug-metal complex. A hypsochromic shift in wavelength of approximately 8–10 nm was observed for the interaction of levofloxacin with Mg^{2+} and enrofloxacin with Cu^{2+}, and 12–16 nm for the interaction of ciprofloxacin HCl with Mg^{2+}. A bathochromic shift of approximately 4–12 nm was observed for the complexation of ciprofloxacin HCl with Fe^{3+}, Al^{3+}, Zn^{2+} and Cu^{2+} ions, and 16–22 nm for the complexation of levofloxacin with Fe^{3+} and Cu^{2+} ions. Large shifts in emission wavelength of drug fluorescence indicate significant changes in the environmental conditions such as hydrophobicities of drugs on interaction with metal ions. Alteration in the hydrophobicities of drugs on metal ion binding has also been reported by Djurdjevic et al. (8). For the interaction of levofloxacin with Al^{3+} and enrofloxacin with Fe^{3+}, Al^{3+} and Mg^{2+} ions, no shift in wavelength was observed. Interaction of levofloxacin and enrofloxacin with Zn^{2+} could not be studied by this method because no change in drug fluorescence was observed.

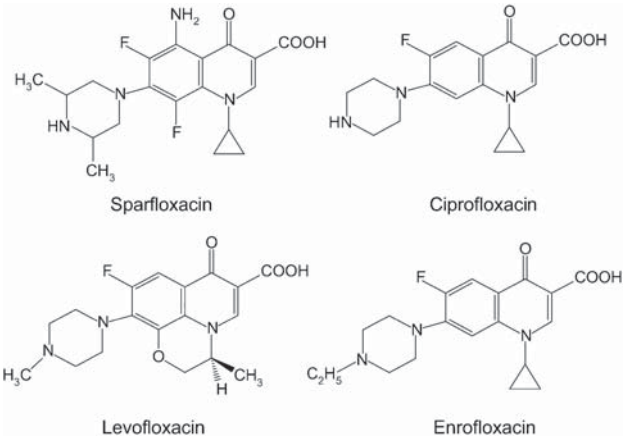

Figure 1 Structures of the studied fluoroquinolones.

Data was analyzed using Eq. [4]. 1/r vs. $1/[M_f]$ plots, called double reciprocal plots, for some representative samples are shown in Figure 2. Association constant (K), for the interaction of fluoroquinolones with metal ions, obtained from the slope of the linear plots, is given in Table 1. Association constants were of the order of 10^4 for the interaction of Fe^{3+}, Al^{3+} and Cu^{2+} and of the order of 10^3 for the interaction of Zn^{2+} and Mg^{2+} for all the fluoroquinolones studied. Association constants of this order indicate somewhat strong interaction of fluoroquinolones with metal ions. For a given drug, the chelation potential of Al^{3+} was highest, whereas that of Mg^{2+} was lowest. Similar observations have also been reported by Ma et al. (6). Percentage of metal ion bound per mole drug, at a metal ion/drug ratio of 1:1 was also calculated from the data. The results are given in Table 2 for various cases. The percentage binding varied widely with the nature of the drug and the nature of the metal cation. In general, the binding affinity was exceptionally low (~9%–16%) for the binding of Mg^{2+}, 26%–29% for the binding of Zn^{2+} and Cu^{2+} to ciprofloxacin HCl and relatively high (50%–73%) in all other cases. The results suggest a significant contribution of metal chelate formation to the antimicrobial activity reduction of fluoroquinolone antibiotics.

Ultra-violet (UV) absorption spectroscopic technique In this method, the absorbance of the drug was determined in the absence and presence of increasing amounts of metal ions. The interaction was accompanied by a red shift in λ_{max} of approximately 1–4, 2–6, 2–10 and 2–5 nm in the case of ciprofloxacin HCl, enrofloxacin, sparfloxacin and levofloxacin, respectively for the various metal cations, again indicating formation of a drug-metal complex. Data was analyzed at λ_{max} of the drug using the Benesi-Hildebrand equation (Eq. [5]). The association constants K, obtained from the slope and intercept of the linear $1/\Delta A$ vs. $1/[M_t]$ plots (Figure 3), are given in Table 3. In the case of Fe^{3+}, data could not be analyzed by this method because the Benesi-Hildebrand plots were curved (Figure 4). Association constants were found to be of the order of 10^4 for Cu^{2+} and Al^{3+}, 10^3 for Zn^{2+} and 10^2 for Mg^{2+} ions.

On comparing the data obtained by fluorescence and UV techniques, it was found that for a given drug, binding affinities for different metal ions varied in the same manner and the order of binding constants were also the same in most cases. The difference in the magnitude of binding constants in some cases can be due to different metal ion concentrations used

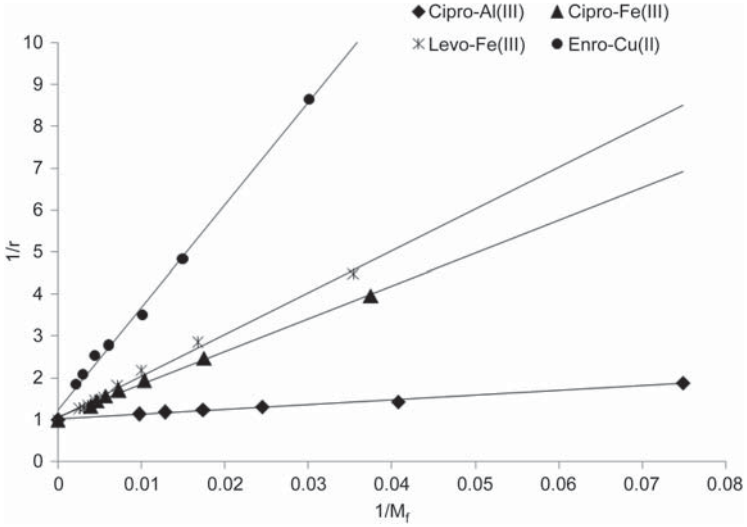

Figure 2 Double reciprocal plots (1/r vs. $1/[M_f]$) for drug-metal ion interaction for some representative cases.

Table 1 Association constants for the binding of fluoroquinolones with metal ions using the fluorescence technique.

Drugs	Association constant (K)×10⁴ M⁻¹				
	Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
Levofloxacin	1.059	2.459	–	2.410	0.124
Ciprofloxacin HCl	1.227	8.434	0.420	0.968	0.132
Enrofloxacin	1.112	1.984	–	1.054	0.541

Table 2 Percentage of metal ion bound per mole drug, at a metal ion/drug ratio of 1:1 for drug-metal ion binding.

Drugs	Percentage of metal ion bound per mole drug $\frac{[M_b]}{[D_t]}\times 100$				
	Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
Levofloxacin	50.6	64.4	–	61.2	10.9
Ciprofloxacin HCl	51.0	72.8	26.5	28.6	8.9
Enrofloxacin	53.3	61.6	–	53.3	15.7

and the inherent difference in the two techniques. For the binding of Al^{3+} to levofloxacin and Cu^{2+} to ciprofloxacin HCl, there was practically no difference in the binding constants determined by the two techniques. For the binding of Cu^{2+} and Al^{3+} to enrofloxacin, the binding constants determined by UV technique were higher. In all other cases, the magnitudes of association constants determined by the UV technique were lower as compared to the fluorescence technique. This can be due to two reasons. Because much higher metal ion concentrations had to be used in the UV method to obtain a measurable change in absorbance, it appears that some low-affinity sites are also involved, and therefore the binding constants are lower. Also, in general, the association constants obtained by the fluorescence technique involves only high-affinity site and are higher, whereas those obtained by the UV technique are the average of the high- and low-affinity sites and are relatively lower.

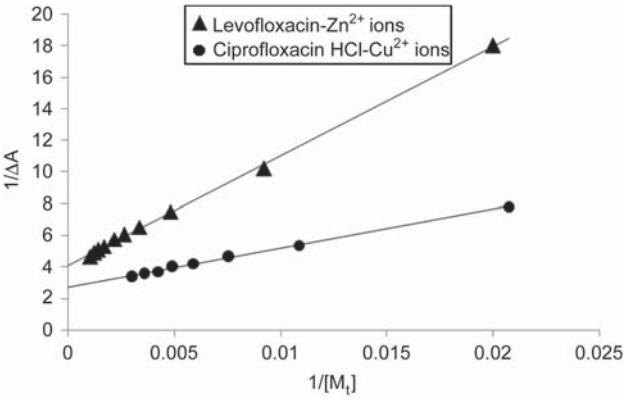

Figure 3 $1/\Delta A$ vs. $1/[M_t]$ plots for the interaction of ciprofloxacin with Cu^{2+} and levofloxacin with Zn^{2+} ions.

Protein-metal ion interaction

HSA-metal ion interaction was accompanied by a blue shift in λ_{max} of 4–8 and 4–16 nm, respectively, for Fe^{3+} and

Table 3 Association constants for the binding of fluoroquinolones with metal ions using the UV spectroscopic technique.

Drugs	Association constant (K)×10³ M⁻¹				
	Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
Levofloxacin	–	26.631	5.924	12.212	0.289
Sparfloxacin	–	29.620	4.708	10.090	0.289
Ciprofloxacin HCl	–	41.451	1.526	10.850	0.225
Enrofloxacin	–	27.392	1.727	29.233	0.325

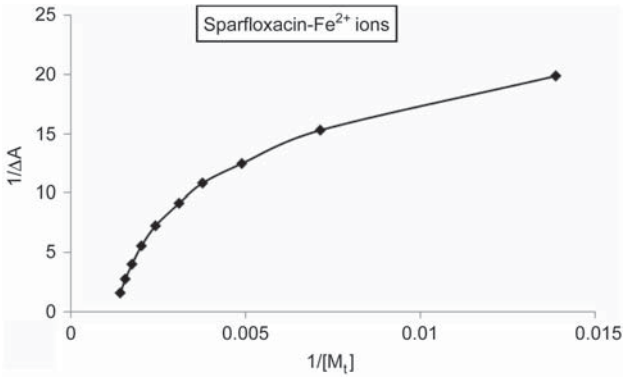

Figure 4 1/ΔA vs. 1/[M_t] plot for interaction of sparfloxacin with Fe^{3+} ions.

Al^{3+}, and a red shift of 4–8 nm for the binding of Cu^{2+}. No shift in λ_{max} was observed for the binding of Zn^{2+} and Mg^{2+} with HSA. The shift in λ_{max} is associated with change in the environment of the metal ion binding site in HSA. Stoichiometry of interaction was assumed to be 1:1 (10) and the association constants for the interaction were calculated using Eq. [4]. Some representative double reciprocal (1/r vs. 1/[M_f]) plots are shown in Figure 5. Mg^{2+} ions did not cause any change in the fluorescence of HSA and therefore association constant for Mg^{2+} could not be determined by this method. For the interaction of Zn^{2+} and Fe^{3+} ions, the plots were linear in the entire concentration range studied and the corresponding association constants are given in Table 4. In the case of Cu^{2+} and Al^{3+}, the plots showed curvature indicating the existence of two types of binding sites. As shown in Figure 6, two straight lines with different slopes could be drawn from the data points and the corresponding high-affinity (K_1) and low-affinity (K_2) association constants could be determined (Table 4). Two classes of binding sites have also been reported by Bal et al. (22) for the binding of Cu^{2+} and Zatta et al. (23) for the binding of Al^{3+} to serum albumin. The high-affinity association constants were of the order of 10^3 for all the metal ions, whereas

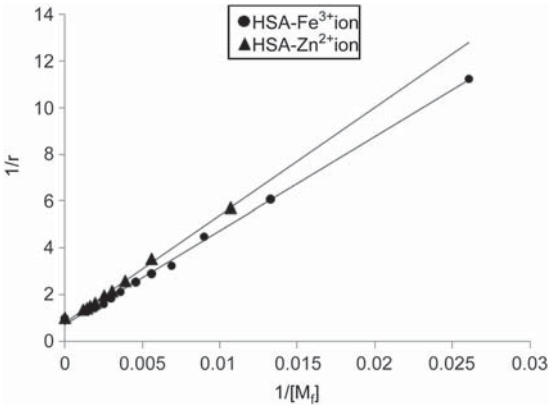

Figure 5 Double reciprocal plot for the interaction of Fe^{3+} and Zn^{2+} ions with HSA at 27°C.

Table 4 Association constants for the binding of HSA with different metal ions.

Metal ions	Association constant (K)×10^3 M^{-1}	
	K_1	K_2
Fe^{3+}	2.614	–
Zn^{2+}	2.327	–
Al^{3+}	9.355	0.817
Cu^{2+}	5.970	0.385

low-affinity binding constants for Cu^{2+} and Al^{3+} were of the order of 10^2.

To obtain a quantitative concept about the magnitude of interaction, data has also been expressed as moles of metal ion bound per mole protein (M_b/P_t)· M_b/P_t (=r) values at metal ion/protein ratios 1 and 5 are recorded in Table 5. The relative affinity of various metal ions was found to vary as Al^{3+}>Cu^{2+}>Zn^{2+}>Fe^{3+}. Thus, the extent of binding is independent of the charge on the ion. Masuoka and Saltman (12) have also reported that HSA has higher affinity for Cu^{2+} ions than Zn^{2+} ions.

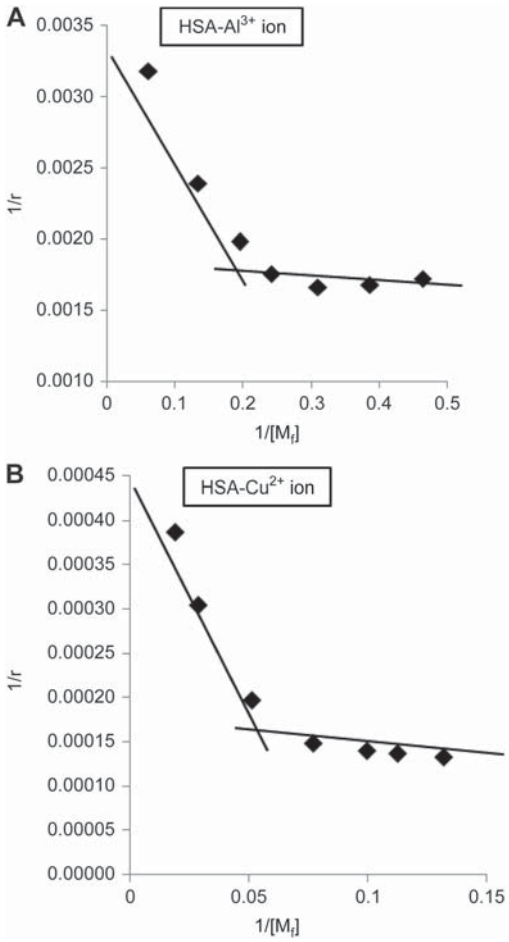

Figure 6 Double reciprocal plots.
(A) Double reciprocal plot for the interaction of Al^{3+} with HSA at 27°C. (B) Double reciprocal plot for the interaction of Cu^{2+} ions with HSA at 27°C.

Table 5 M_b/P_t values at different metal ion/protein ratios for HSA-metal ion interaction.

M_t/P_t	Moles of metal ion bound per mole protein (M_b/P_t)				
	Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
1	0.141	0.452	0.162	0.275	–
5	0.166	0.541	0.177	0.314	–

Drug-protein interaction in the presence of metal ions

Drug-HSA interaction in the presence of metal ions was accompanied by a red shift (approximately 4–16 nm) in the fluorescence emission λ_{max} of protein in most cases. However, because fluoroquinolone-HSA interactions in the absence of metal ions are also accompanied by shift in λ_{max}, it is the net shift ($\Delta\lambda_{D\text{-}P\text{-}M} - \Delta\lambda_{D\text{-}P}$), the difference between the shift in the presence ($\Delta\lambda_{D\text{-}P\text{-}M}$) and absence ($\Delta\lambda_{D\text{-}P}$) of metal ions, which is more important. The net shift produced due to the presence of metal ions for various fluoroquinolones is given in Table 6. No net shift was observed in the presence of Al^{3+} and Mg^{2+} ions in all cases except enrofloxacin. In the case of enrofloxacin, a blue shift of 4–10 nm was observed. In the presence of Fe^{3+}, a red shift (4–8 nm) was observed in all cases except levofloxacin, where no change was observed. In the presence of Cu^{2+}, no shift was observed for ciprofloxacin HCl and enrofloxacin; 4–8 nm blue and red shift, respectively, was observed for levofloxacin and sparfloxacin. In the presence of Zn^{2+} ions, the binding of levofloxacin and ciprofloxacin HCl was accompanied by a blue shift (4–8 nm); a red shift (4–8 nm) was observed for enrofloxacin, whereas no shift was observed in the case of sparfloxacin. Thus, the observed shift could not be generalized. Red shift indicates decrease in hydrophobicity, whereas blue shift indicates increase in hydrophobicity of microenvironment of fluoroquinolone binding sites on HSA.

The binding data has been reported in terms of association constants in the absence and presence of metal ions and change in the percentage of free drug due to the presence of metal ions. Association constant data are given in Table 7. The association constants were of the order of 10^4 in most cases. The presence of Cu^{2+} ions was found to increase the binding affinity of all the fluoroquinolones to HSA. Tan et al. (16) have also shown increase in the HSA-drug association constant in the presence of Cu^{2+} ions for gatifloxacin. All other metal ions (Zn^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}) decreased the binding of fluoroquinolones to HSA, with the exception of levofloxacin in the presence of Zn^{2+} and Al^{3+} ions. Bi et al. (24) have also reported that the binding constants for the interaction between tetracyclines and serum albumin decreased in the presence of Zn^{2+}, Mg^{2+} and Ca^{2+} but increased in the presence of Cu^{2+} ions.

To interpret the results, we have to keep in mind that several competing reactions are possible in the HSA-metal-fluoroquinolone system. For example, (i) HSA can interact with metal ion through high-affinity metal binding sites; (ii) fluoroquinolones are also known to form somewhat strong drug-metal chelates; and (iii) HSA can also interact with the fluoroquinolones.

To what extent HSA interacts with fluoroquinolone in the presence of metal ions depends on the relative affinity of HSA as well as fluoroquinolone for metal ions. Thus, there are two possible modes of interaction: either the HSA-metal ion complex interacts with the drug or the drug-metal complex interacts with HSA. Further drugs can displace metal ions from binding sites on HSA, and HSA can also be conformationally altered due to the formation of the ternary HSA-drug-metal ion complex.

As discussed in the previous sections, Cu^{2+} has high affinity for both HSA and fluoroquinolones. Increase in HSA-fluoroquinolone association constant in the presence of Cu^{2+} ions indicates that metal ions facilitate HSA-fluoroquinolone interaction. Fluoroquinolones probably interact with HSA via a metal ion bridge (17). Same explanation could hold for the increase in HSA-levofloxacin association constant in the presence of Zn^{2+} and Al^{3+} ions. Decrease in association constants in other cases cannot be due to competitive interference because metal binding sites are usually distinct from the fluoroquinolone binding sites (12). Because all these metal cations have somewhat strong interaction with fluoroquinolones, the formation of the fluoroquinolone-metal ion complex can affect or inhibit the fluoroquinolone-HSA interaction. Decrease in binding constants can also be due to conformational changes in the albumin molecule. Although the fluoroquinolone and metal ion binding sites might not be in the same domain, formation of the HSA-metal ion-fluoroquinolone ternary complex could lead to conformational changes in the albumin.

Higher binding constants in the presence of metal ions result in prolonged storage time of the drug in blood plasma, whereas concentration of free pharmacologically active drug

Table 6 Net shift, the difference between the shift in the presence and absence of metal ions.

Metal ion	Wavelength shift[a]			
	Levofloxacin	Sparfloxacin	Ciprofloxacin HCl	Enrofloxacin
Absence of metal ions	+ (4–8) nm	– (4–8 nm)	+ (4–8) nm	+ (8–10 nm)
Fe^{3+}	No change	+ (4–8 nm)	+ (4–8 nm)	+ (4–8 nm)
Al^{3+}	No change	No change	No change	– (4–8 nm)
Zn^{2+}	– (4–8 nm)	No change	– (4–8 nm)	+ (4–8 nm)
Cu^{2+}	– (4–8 nm)	+ (4–8 nm)	No change	No change
Mg^{2+}	No change	No change	No change	– (8–10 nm)

[a]–symbol indicates shift towards lower wavelength (blue shift) and + symbol indicates shift towards higher wavelength (red shift).

Table 7 Association constants for the binding of fluoroquinolones with HSA in the absence and presence of metal ions.

Drugs	Association constant (K)×10^4 M^{-1}					
	Absence of metal ions	In the presence of				
		Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
Levofloxacin	9.050	8.975	12.273	18.405	14.006	6.904
Sparfloxacin	10.550	3.844	2.913	8.023	11.801	4.367
Ciprofloxacin HCl	5.780	3.208	4.559	4.618	7.086	3.863
Enrofloxacin	4.620	0.833	1.616	3.088	8.506	0.832

Table 8 Percentage of free drug in the absence and presence of metal ions at D_t/P_t ratio of 0.5.

Drug	Percentage of free drug (α)					
	Absence of metal ions	In the presence of				
		Fe^{3+}	Al^{3+}	Zn^{2+}	Cu^{2+}	Mg^{2+}
Levofloxacin	56.8	58.8	51.2	43.0	48.5	63.5
Sparfloxacin	54.6	75.2	79.0	60.8	53.6	71.6
Ciprofloxacin HCl	66.9	78.1	71.8	71.5	63.0	73.8
Enrofloxacin	71.4	92.7	86.7	78.0	60.0	92.7

decreases. Decrease in binding constants, by contrast, increases the concentration of free drug which could result in toxic reaction or quick clearance from blood.

The percentage of bound drug (β) and the percentage of free drug (α) were also calculated from the association constant data (19, 20). The percentage of bound drug (β) vs. drug/protein ratio plots for one representative fluoroquinolone (ciprofloxacin HCl) in the absence and presence of metal ions are shown in Figure 7. For all the studied drugs, α values in the absence and presence of various metal ions, at D_t/P_t ratio of 0.5, are given in Table 8. Significant change in the percentage of free drug was observed especially at low drug/protein ratios which are encountered in the physiological system. For example, at a drug/protein ratio of 0.5, percentage of free sparfloxacin increased from 54.6% to 79% in the presence of Al^{3+} ions, percentage of free levofloxacin decreased from 56.8% to 43% in the presence of Zn^{2+} ions, percentage of free ciprofloxacin increased from 66.9% to 78.1% in the presence of Fe^{3+} ions, percentage of free enrofloxacin increased from 71.4% to 92.7% in the presence of Mg^{2+} and Fe^{3+} ions.

Conclusions

Drug-metal ion, HSA-metal ion and drug-HSA interaction in the absence and presence of metal ions have been studied using spectroscopic techniques. For fluoroquinolone-metal ion interaction, in general, the binding affinity was exceptionally low (~9%–16%) for the binding of Mg^{2+}, 26%–29% for the binding of Zn^{2+} and Cu^{2+} to ciprofloxacin HCl and relatively high (50%–73%) in all other cases. The results provide a quantitative concept about the effect of different metal cations on reduction in antimicrobial activity of fluoroquinolone antibiotics. For HSA-metal ion interaction, the high-affinity association constants were of the order of 10^3 for all the metal ions, whereas low-affinity binding constants for Cu^{2+} and Al^{3+} were of the order of 10^2. The relative affinity of various metal ions was found to vary as Al^{3+}>Cu^{2+}>Zn^{2+}>Fe^{3+}. The presence of metal ions also had a significant effect on the drug-HSA interaction. The binding affinity of all the studied fluoroquinolones to HSA increased in the presence of Cu^{2+} ions. All other metal ions (Zn^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}) decreased the binding of fluoroquinolones to HSA, with the exception of levofloxacin in the presence of Zn^{2+} and Al^{3+} ions. The increase/decrease in binding affinity has been quantitatively expressed in terms of change in the percentage of free drug due to the presence of metal ions. The competitive binding mechanism involved and the possible effect of metal ions on drug pharmacology has been discussed.

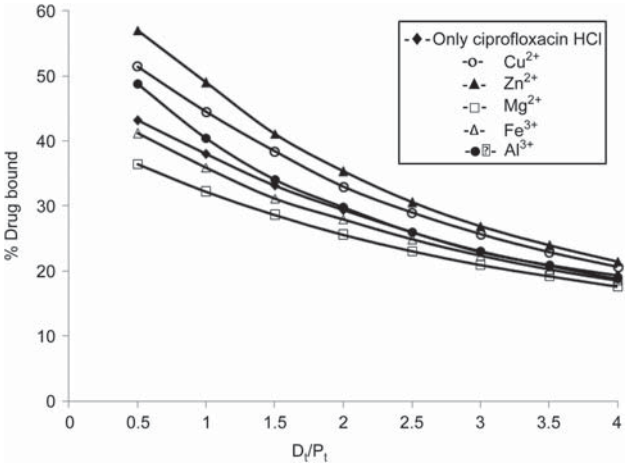

Figure 7 Percentage of ciprofloxacin HCl bound to HSA in the absence and presence of metal ions.

Conflict of interest statement

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