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ORIGINAL PAPER

Influence of Mg^{+2} and Cu^{+2} on the Interaction Between Quinolone and Calf Thymus DNA

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Abstract Mg^{+2} and Cu^{+2} have different binding capacities to quinolone drugs and have different binding modes with calf thymus DNA. Using the method of absorption and fluorescence spectroscopy, the influence of Mg^{+2} and Cu^{+2} on the binding between calf thymus DNA and each of four quinolone drugs has been studied. The results show that both Mg^{+2} and Cu^{+2} can bind with the four drugs. In the absence of divalent metal ions, quinolone drugs interact with DNA double helix by forming hydrogen bonds between the carboxyl and carbonyl groups of the drugs and the phosphate groups of the DNA bases, and the binding capacity shows a close relationship with the drug structures. The two metal ions show different influences on the binding between the drug and DNA, which depends on the type of ion, concentration of the metal ions and the structure of drugs. Mg^{+2} in lower concentrations (0.01 mM to 3.0 mM) can act as a bridge between the carboxyl group/carbonyl group of the drug and the phosphate group of the DNA by electrostatic interaction, while Cu^{+2} can act as an intermediary ion between carboxyl group/carbonyl group of the drug and the DNA bases by a co-ordinate bond. Both actions can increase the interaction of the π electron between the condensed rings of the drugs and the DNA bases. In some conditions, Cu^{+2} can weaken the binding between the drug and the DNA by competitive inhibition if there is a site on the drug that can directly bind both DNA bases and Cu^{+2}.

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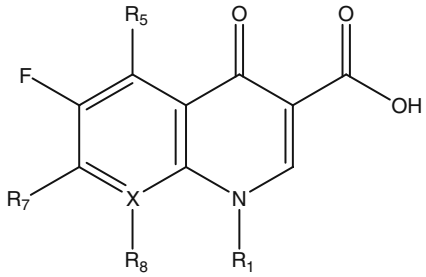
Keywords Quinolone drugs, Calf Thymus DNA, Mg^{+2} · Cu^{+2}

Introduction

The term quinolones is commonly used for the quinolonecarboxylic acid (or 4-quinolones) group of synthetic antibacterial agents, which contain a 4-oxo-1, 4-dihydroquinoline skeleton (Scheme 1). Since the introduction of nalidixic acid into clinical practice in the early 1960s, a number of structurally-related highly potent broad-spectrum antibacterial agents have been isolated [1, 2].

Quinolones are known to inhibit the action of essential bacterial enzymes, including DNA gyrase and DNA topoisomerase IV, which are both sensitive to the 4-quinolone class of antibacterial compounds in vitro. To clarify the mechanism of quinolone action, studies have been conducted on the direct binding of quinolones to DNA or DNA gyrase, which are the logical potential targets for quinolones. However, contradictory reports have appeared in the literature on molecular details of drug-DNA and drug-enzyme interactions [3–5]. Although the exact mechanism of action remains unclear, there is evidence that quinolones interact directly with DNA in synergy with the gyrase enzyme [3, 6]. Such an interaction undoubtedly contributes to the antibacterial activity; however, it may also be responsible, at least partially, for the unwanted toxic side-effects [7]. Therefore, for a better understanding of their therapeutic efficacy, a deeper insight into the interaction mechanism of this antibiotic class with DNA is important.

Shen and co-authors proposed a drug-DNA model that implied hydrogen-bond type interactions between unpaired DNA bases and the quinolone, as well as a stacked dimerization of the drug [8]. The model has been modified



Scheme 1 Quinolone skeleton

to include a possible interaction between the C-7 substituent and the quinolone pocket on the B subunit of DNA gyrase [9]. Palumbo et al. stressed the role of magnesium in the quinolone-DNA interaction [10]. It was suggested that Mg^{+2} acts as a bridge between the quinolone and the phosphate group of the DNA, and that this complex is stabilized by stacking interactions between the condensed rings of the drug and the DNA bases in a single-stranded region or a distorted B-form in a plasmid. In a subsequent study it was confirmed that affinity of the quinolone for DNA, modulated by Mg^{+2}, plays an important role in poisoning the cleavable gyrase-DNA complex and, consequently, in eliciting antibacterial activity by this family of drugs [11]. The results obtained from different 6-substituted compounds support the idea that position 6 of the drug, aside from playing a pharmacokinetic role, is involved in recognition of the enzyme pocket [11]. Liorente et al. proposed another model based on the interaction of quinolone with the double helix of DNA [12], and they concluded that the drug-DNA complex is stabilized by the binding of a magnesium ion with the SP^2 oxygen present in quinolone, a phosphate and a purine base of the DNA. Substituents R_1 and R_7 are predicted to make hydrophobic interactions in the major and minor groove of DNA, respectively. R_7 could also form hydrogen bonds with amino groups of guanines and the aspartic acid residue at position 87 in DNA gyrase subunit A.

We can conclude that the mode of action of these drugs and related processes were extensively studied in the past. However, the topic is extremely important due to the fact that several quinolones are used in clinical practice and their exact mechanism of action remains to be elucidated [11].

Recent studies have proposed the important role of metal ions in the mechanism of action of these drugs. From a theoretical-experiment study on the structure and activity of certain quinolones and the interaction of their Cu^{+2} complexes on a DNA model, it was suggested that the intercalation of the quinolone complexed to a metal is an important step in the drug's activity [13].

The proposed mechanism of the interaction between quinolone and metal cations is the chelation between the metal and the 4-oxo and adjacent carboxy groups. These functional groups are required for antibacterial activity, therefore it could be anticipated that all of the quinolones will interact with metal ions, however, some authors proposed that there might be differences between the quinolones regarding the extent of interaction [14]. Our previous studies have shown that, under the same conditions, certain metal ions have different interactions with quinolones and have different influences on the binding between the drugs and DNA [15–17].

In this study, four quinolones, Ciprofloxacin (CPFX), Fleroxacin (FLRX), Gatifloxacin (GTFX) and Sparfloxacin (SPFX), which have different structures, have been selected and their interactions with DNA in the absence and presence of Cu^{+2} and Mg have been studied using UV–absorption and fluorescence spectroscopy. Based on the experimental results, an interaction model of quinolone with DNA and the action of metal ions have been discussed further, and a possible structure of the quinolone-DNA complex has been proposed.

Materials and methods

Materials

CPFX, FLRX, GTFX and SPFX (LKT labs, Inc, Japan) were kept as solids in the dark at 4°C. A 0.02 M NaCl stock solution of above drugs was prepared immediately prior to use, and the concentration of these drugs remained 1.0×10^{-3} M. Mg^{+2} and Cu^{+2} (as $MgCl_2$ and $CuCl_2$) were obtained from Tianjin Chemical Reagents Company of China, and the concentrations of Mg^{+2} and Cu^{+2} in the 0.02 M NaCl drug stock solutions were 1 M and 0.1 M, respectively. Calf thymus DNA (Sigma) was prepared by dissolving the DNA in a 0.02 M NaCl solution. DNA concentrations were expressed as DNA phosphate (DNA P) with a molar extinction coefficient of 6,600 M^{-1} cm^{-1} at 260 nm [17].

Methods

The absorption and fluorescence spectra were obtained using a UV-2450 spectrophotometer and an RF-5301PC fluorescence spectrophotometer (Shimdazu, Japan). In fluorescence mode, both excitation and emission bandwidths were set at 10 nm. All of the spectroscopic work was carried out at ambient temperature and at pH 7.0 in Tris–HCl buffer (0.01 M).

Each drug–DNA, drug–Mg^{+2} and drug–Cu^{+2} binary system was formed by titrating the drug solution with DNA, $MgCl_2$ and $CuCl_2$ solutions separately. The concentration of each drug was maintained at 1.0×10^{-5} M in absorption spectra and fluorescence spectra determinations. Each drug–DNA–Mg^{+2} and drug–DNA–Cu^{+2} ternary system was formed by titrating the drug–DNA binary system with Mg^{+2} and Cu^{+2} solutions. The molar ratios of drug to DNA were set to vary from 0.07 to 0.4. During the titration

operations, DNA, $MgCl_2$ and $CuCl_2$ stock solutions were added in microliter quantities [17].

Results and discussion

Binary binding of drug with Mg^{+2}, Cu^{+2} and DNA

For the absorption spectra of the drug solutions are shown in Table 1. Titrating drug solutions with Mg^{+2}, Cu^{+2} and DNA in increasing amounts separately caused a decrease in absorbance associated with the chromophore of the drugs. As an example, titrating a GTFX solution with Mg^{+2}, Cu^{+2} and DNA is shown in Fig. 1. These results indicate that the drugs can bind with Mg^{+2}, Cu^{+2} and DNA, and that the bound chromophores are converted to new species.

Under the aggregation conditions, drugs were fixed at 1.0×10^{-5} M and the titrated DNA varied from 1.3×10^{-4} to 6.5×10^{-4} M. The relative fluorescence intensity (RFI) of drugs was determined. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) applied in the determination are shown in Table 1. The titrations markedly enhanced (for Mg^{+2}) or quenched (for Cu^{+2} or DNA) the RFI of the drugs. Figure 2 illustrates the RFI change of the GTFX solution titrated with Mg^{+2}, Cu^{+2} and DNA.

Referring to the changes of absorbance of the same system, it can be confirmed that the ground state reaction between each drug with Mg^{+2}, Cu^{+2} and DNA has taken place. The binding constant of each drug with Mg^{+2}, Cu^{+2} and DNA can be calculated by the following equations [18, 19]:

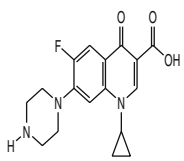
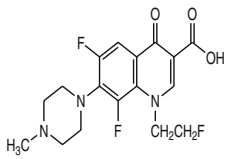
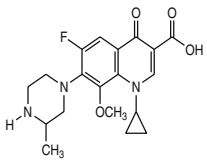
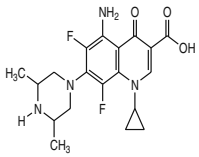
$$(F_{cm}-F_c)^{-1} = K_a^{-1}\cdot K_i^{-1}Q_{cm}^{-1}[C]_o^{-1}\cdot[M]_o^{-1} + K_i^{-1}Q_{cm}^{-1}[C]_o^{-1} = \mathrm{B}\cdot[\mathrm{M}]_\mathrm{o}^{-1} + \mathrm{A}(\text{For RFI enhancement}) \quad (1)$$

$$\lg[(F_0-F)/F] = \lg K + n\lg[Q] \quad (\text{For RFI quenching}) \quad (2)$$

In Eq. 1, (F_{cm}-F_c) is the change of RFI of drug before and after adding sensibilizer; $[M]_o$ is the concentration of sensibilizer; $[C]_o$ is initial concentration of drug; Q_{cm} is the quantum yield of drug-sensibilizer complexes; K_i is the instrument constant. A curve can be constructed by $1/(F_{cm}-F_c)$ versus $1/[M]_o$, and the binding constant of drug with sensibilizer (K) can be obtained by B/A. In Eq. 2, F is the RFI of free drug in the system; F_0 is the RFI of the total drug; [Q] is the equilibrium concentration of the quencher; K is binding constant of the drug with the quencher; n is the binding site of quencher with the drug.

When each drug solution was titrated with Mg^{+2}, Cu^{+2} and DNA, the change of RFI was determined, and the K, n

Table 1 Binary binding of drugs with Mg^{2+}, Cu^{2+} and DNA

Drug		Ciprofloxacin	Fleroxacin	Gatifloxacin	Sparfloxacin
Structure					
λ_{ABS}(nm)		280, 330	280, 330	285, 340	290, 370
$\lambda_{ex}/\lambda_{em}$(nm)		330/420	330/450	340/450	370/510
Drug-Mg^{+2}	RFI	↑	↑	↑	↑
	K	657	21	7.9	7558
	n*	1:1	1:1	1:1	1:1
Drug-Cu^{+2}	RFI	↓	↓	↓	↓
	K	21038	4219	6280	14489
	n	1	1	1	1
Drug-DNA	RFI	↓	↓	↓	↑
	K	179	322	506	5662
	n (n*)	1	1	1	1:1

↑ represent RFI enhancement, ↓represent RFI quenching; K: represent binding constant; n: represent binding site; n*: represent complexation ratio

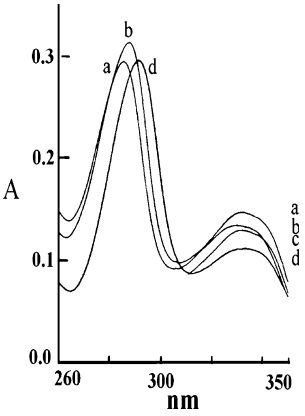


Fig. 1 Absorbance changes that occur when the GTFX is titrated with Mg^{2+}, Cu^{2+} and DNA: pH 7.0; C_{NaCl}(M): 0.02; C_{GTFX} (M): 1.0×10^{-5}; C_{Mg}(M): 2.0×10^{-3}; C_{Cu} (M): 3.33×10^{-4}; C_{DNA} (M): 1.2×10^{-4}; **a** GTFX; **b** GTFX+Mg^{+2}; **c** GTFX+DNA; **d** GTFX+Cu^{+2}

values and n^* were calculated by the equations above. The results are shown in Table 1.

The results in Table 1 raise some interesting discussion points.

(1) Differently from the other drugs, SPFX has an absorbance peak at 370 nm, which shifts to a lower energy compared with other drugs (330~340 nm). The change can be attributed to the action of the amino group introduced into SPFX. Amino groups, which are electron-donating groups, can extend the conjugated double bonds system of the drug and cause the absorbance peak of SPFX shift to a longer wavelength.

(2) The bonding of Mg^{+2} to the drugs increases the fluorescence intensity of the drugs, while for Cu^{+2}; the bonding can quench the fluorescence intensity. This phenomenon can be explained as follows: Mg^{+2} chelates with the carboxyl group and carbonyl groups of the drugs, and this action helps to form a larger conjugate system in the drugs and to stabilize the rigid structure of the drug plane. Consequently, the fluorescence intensity increases. On the other hand, Cu^{+2} can quench the fluorescence in most conditions because of its electron characteristic of paramagnetism as a transition metal. Therefore when the drugs chelate with Cu^{+2}, the fluorescence intensity decreases.

(3) The binding of SPFX to DNA can increase the fluorescence intensity of the drug. This suggests that the binding of SPFX with DNA may have a different mode compared with the other drugs in the experiment. A possible mechanism is that the amino group in SPFX participates in the binding between the drug and DNA. As a result, a larger rigid plane of the conjugated system in the drug has been formed, consequently the fluorescence intensity of the drug increases.

(4) CPFX shows a stronger binding capacity with Mg^{+2} and Cu^{+2}. FLRX and GTFX both show weaker binding capacity with Mg^{+2} and Cu^{+2} owing to the introduction of a fluorine group (-F) (for FLRX) and a methoxy group (-OCH_3) (for GTFX) at C_6 in the drugs (both the groups at the drugs belong to the electron-withdrawing group, so the introduction of these groups into the drugs can weaken the negative radical at the carboxyl group (-COOH) and carbonyl group (-CO-) of the drugs). For SPFX, owing to the introduction of an amino group (-NH_2) at C_5 (which can enhance the negative radical at the carboxyl group and the carbonyl group in the drug, which action helps improve the formation of hydrogen bond between oxygen at these groups and bases in DNA), it shows a stronger binding capacity with Mg and Cu^{+2}, especially for Mg^{+2}, than the other drugs.

(5) Another possible mechanism for the stronger binding of SPFX is that the introduced amino group in SPFX in some way participates directly in the binding between the drugs with the DNA and these results in a stronger binding capacity between them.

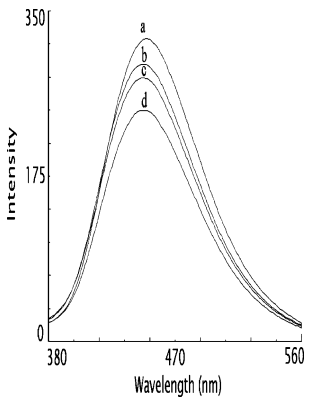


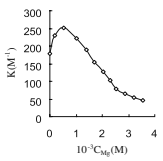
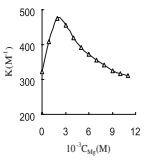
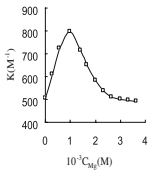
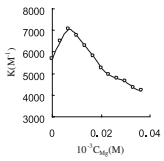
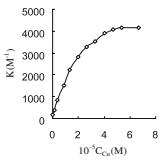
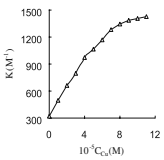
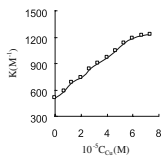
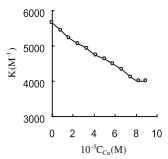
Fig. 2 Fluorescence changes that occur when the GTFX is titrated with Mg^{2+}, Cu^{2+} and DNA: pH 7.0; C_{NaCl} (M): 0.02; λ_{ex}=340 nm. C_{GTFX} (M): 1.0×10^{-5}; C_{Mg}(M):3.0×10^{-3}; C_{Cu} (M): 3.0×10^{-5}; C_{DNA} (M): 1.3×10^{-4}. **a** GTFX+Mg^{+2}; **b** GTFX; c: GTFX+DNA; d: GTFX+Cu^{+2}

The influence of Mg^{+2} and Cu^{+2} on the binding between Quinolones and DNA

Table 2 illustrates the influence of Mg^{+2} and Cu^{+2}, respectively, on the changes in the binding constants between quinolones and calf thymus DNA. The main points to note from these results are:

(1) The influence of Mg^{+2} on the binding between quinolones and DNA has a similar action for the four drugs applied in the experiment. At lower Mg^{+2} concentrations (0.01 mM to 3.0 mM), Mg^{+2} can increase the binding constants between the drugs and DNA. However when the Mg^{+2} concentration in the system increases further, the binding between drugs and the DNA weakens. A possible mechanism for this is that at lower Mg^{+2} concentrations, Mg^{+2} can act as a bridge between the carboxyl group and the carbonyl group of the quinolone and the phosphate group of the

Table 2 The influence of Mg^{2+} and Cu^{2+} on the binding constants between drugs and DNA

System	CPFX–DNA	FLRX–DNA	GTFX–DNA	SPFX–DNA
Titrating with Mg^{+2}				
Titrating with Cu^{+2}				

DNA, and that this complex can be stabilized by stacking interactions of the π electron between the condensed rings of the drugs and the DNA bases. All four drugs have the carboxyl group and carbonyl group at C_3 and C_4 positions, therefore Mg^{+2} shows a similar action in that it can increase the binding between the drugs and the DNA. As the Mg^{+2} concentration increases, it is possible for Mg^{+2} to produce a salt effect, which can weaken the interaction of the π electron between the condensed rings of the drugs and the DNA bases, and as a result, the binding constants between the drugs and the DNA begins to decrease.

(2) It can be seen from Table 2 that as Cu^{+2} concentrations increase in the binary systems, the binding constants between CPFX, FLRX, GTFX (which all have no amino group in their structure) and DNA increased, while the binding constant between SPFX and DNA decreased. This illustrates that the former three drugs can bond with DNA by the intermediary action of Cu^{+2} between the carboxyl group and the carbonyl group of the drugs and DNA bases[20]. For SPFX, in addition to the interaction between the carboxyl group, carbonyl group and the phosphate groups of the DNA bases, the amino group at C_5 of SPFX perhaps bonds directly with a position of a DNA base which can also bind with Cu^{+2}, therefore SPFX shows a higher binding constant compared with the other three drugs. However, the binding between the amino group of SPFX and the DNA base might also be influenced competitively by Cu^{+2}, accounting for gradual decrease in the binding constant between SPFX and DNA as the Cu^{+2} concentration increases in the SPFX-DNA binary system,.

Conclusions

(1) In the absence of divalent ions, quinolone drugs can interact with the DNA double helix by forming hydrogen bonds between the carboxyl groups and the carbonyl groups of the drugs and the phosphate groups of the DNA bases. The complex is stabilized further by the interaction of the π electron between the condensed rings of the drugs and the DNA bases. The binding capacity of the drugs with DNA has a close relationship with the structure of the drugs. When an electron-donating group such as amino group is introduced into the C_5 position of quinolone, the binding between drugs and DNA increased markedly. The possible mechanism is that the introduced electron-rich group can bind directly with DNA bases so as to enhance the interaction of the π electron between the condensed rings of the drugs and the DNA bases.

(2) Mg^{+2} can act as a bridge between the carboxyl group, the carbonyl group of drug and the phosphate group of the DNA. Over a certain concentration range (0.01 mM to 3.0 mM), Mg^{+2} can stabilize the binding between the drug and DNA and increase the interaction of the π electron between the condensed rings of the drug and the DNA bases.

(3) Cu^{+2}can act as an intermediary ion between the carboxyl group and the carbonyl group of the drug and the DNA bases, and further stabilize the interaction of the π electron between the condensed rings of the drug and the DNA bases. Increasing the concentration of Cu^{+2} over a certain range can promote the binding between the drug and DNA.

(4) SPFX contains an amino group that can bind directly with the DNA. Having a binding site in the DNA bases, this special group can also be bound by Cu^{+2}. So if Cu^{+2} presents in the SPFX-DNA binary system, it can weaken the binding between SPFX and the DNA and further weaken the interaction between the condensed rings of the drug and the DNA bases.

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