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The Interaction Between Levofloxacine Hydrochloride and DNA Mediated by Cu^{2+}

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Levofloxacin (LEV) is a fluoroquinolone antimicrobial agent. LEV also inhibits DNA synthesis and is bactericidal. The mechanisms of the interaction among LEV, DNA and Cu^{2+} are studied by fluorescence method. In the paper, the results show that LEV and Cu^{2+} can form binary complex, and also LEV and DNA can form complex mediated by Cu^{2+}. The composition of the complex is determined. The affects to the reaction are also found.

KEY WORDS: Levofloxacin; DNA; Cu^{2+}; fluorescence.

INTRODUCTION

The nature and dynamics of binding small molecules to biomacromolecules represent an active area of investigation. Studies directed towards the design of site- and conformation-specific reagents provide routes towards rational drug design [1,2,3,4].

Fluoroquinolone develops its pharmacological action via specific inhibition of sub-unit A of the bacterial gyrase, an enzyme that controls DNA shape [5]. Although the exact mechanism of this action is still unclear, there is evidence that fluoroquinolone interacts directly with DNA in synergy with the gyrase enzyme [6,7]. Such interaction undoubtedly contributes to the desired antibacterial activity but it can also be responsible, at least in part, for the unwanted toxic effects. Contributions to deeper insight into the mechanism of interaction of this class of antibiotics with DNA might be important for a better understanding of their therapeutic efficacy.

The interest of this field is diverse; since metal ions were found play an important role in the DNA and RNA structure. They realized that the major difference in the experimental conditions between their work and that of Shen and his coworkers was Mg^{2+} concentration. Later, gelelectrophoresis experiments by Tornaletti and Pedrini [8] showed that ciprofloxacin is able to unwind the DNA double helix in the presence of Mg^{2+}. Sophie [9] used NMR spectra and the results show that in the presence of magnesium, pefloxacin binds strongly to DNA. Wu [10] studied the fluorescence spectra of fluoroquinolone antibiotics including ofloxacin, norfloxacin and ciprofloxacin complexed with Co^{2+}, the fluorescence spectral data appears that the fluoroquinolone antibiotic cannot directly complex with ATP but indirectly complex with Co^{2+}, which is playing an intermediary role. Yuan [11] used fluorescence method studied the interaction of between ciprofloxacin and DNA mediated by Cu^{2+}, Sissi, Claudia[12] etal studied the interaction of a number of novel 6-substituted quinolone derivatives with DNA in the presence/absence of magnesium ions by fluorometric techniques. T. Skauge[13]studied interaction between ciprofloxacin and DNA mediated by Mg^{2+}.

Levofloxacin (LEV), (−)-(*S*)-9-fluoro-2.3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2,3-de]-1,4-benzoxazine-6-carboxilic acid hemihydrate (Fig. 1), is a fluoroquinolone antimicrobial agent which exhibits broad-spectrum bactericidal activity against gram-positive andgram-negative bacteria (aerobic and anaerobic). Levofloxacin is the pure (−)-(*S*)-enantiomer

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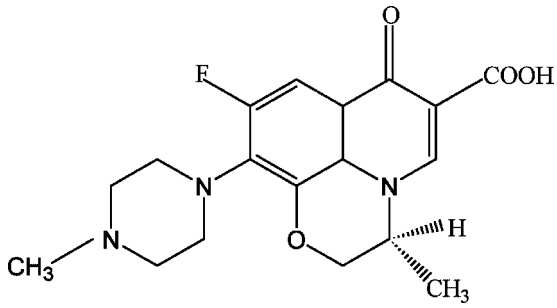

Fig. 1. Structure of LEV.

of the racemic drug substance ofloxacin and is approximately two-fold more potent than the racemic mixture that constitutes the parent drug (8–128-fold more potent than the $R(+)$ enantiomer) [14].

The structure of LEV is shown in Fig. 1. LEV is related to other quinolones including cinoxacin, ciprofloxacin, enoxacin, ofloxacin and nalidixic acid. It is the derivative of 4-quinolone reveals that there is an active grouping in the form of the —COOH and —C═O capable of chelate formation with metal ions [15]. The fluorine atom provides increased potency against Gram-negative organisms and its activity. LEV also inhibits DNA synthesis and is bactericidal [16,17].

In this article, different metal ions are selected to be mediums in the interaction between LEV and DNA. Furthermore, the interaction between LEV and DNA mediated by Cu^{2+} is studied by fluorescence and method.

EXPERIMENTS

Reagents

All reagents for synthesis are of analytical reagent grade unless otherwise stated. Double distilled water is used throughout the work.

The working concentration of LEV (Made in Norman Bethune University of Medical Science) solution is 2.5×10^{-4} mol/L. The stock solution of DNA is prepared by dissolving commercially purchased calf thymus DNA (Baitai Biochemical Co., Chinese Academy of Sciences) in doubly distilled water at 0–4°C. Twenty-four hours or more are needed for dissolving DNA with occasional gentle shaking. The concentrations of stoke solutions of nucleic acids are determined by the absorbance at 260.0 nm. The working concentration of the nucleic acid solution is 6.0×10^{-4} mol $\cdot$ L^{-1}, which is prepared by diluting the stock solution with deionized water. The stock solution of $CoCl_2$, $FeCl_2$, $CuCl_2$, $CuSO_4$ and Cu $(NO_3)_2$ are prepared and the working concentration of them are 1.0×10^{-3} mol $\cdot$ L^{-1}. 0.1 mol $\cdot$ L^{-1} NaCl and 0.1 mol $\cdot$ L^{-1} $MgCl_2$ are used to adjust the ionic strength of the aqueous solutions. The Tris-HCl buffers with different pH are prepared by mixed 0.1 mol $\cdot$ L^{-1} Tris solution and HCl solution.

Apparatus

The fluorescence spectra are measured with a Shimadzu RF-540 spectrofluorometer with a 1×1 cm cross-section quartz cell (Kyoto, Japan). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) is used to blend the solution, and a PHB-4 pH meter is used to measure the pH of the solution. A super thermostat circulating water bath was used for maintaining the different temperatures of the systems for the fluorescence quenching experiments.

Procedures

In a dry 12.5 mL volumetric flask are added 0.2 mL of the LEV solution, 1.0 mL of the $CuCl_2$ solution, 1.0 mL of Tris-HCl solution, and appropriate volumes of nucleic acid. The mixture is diluted to 12.5 mL with doubly distilled water and vortexes. Five minutes later, the fluorescence spectra are measured with the following settings of spectrofluorometer: excitation wavelength (λ_{ex}), 295 nm and 340 nm; emission wavelength (λ_{em}), 460 nm; both the excitation and emission slits are 10 nm. And then all the absorption and fluorescence spectra measurements are obtained against the blank treated in the same way without nucleic acid.

RESULT AND DISCUSSIONS

The Selection of Metal Ion

The fluorescence spectra of LEV and its decreased fluorescence intensity by DNA and different metal ions are present in Fig. 2. The spectra show that LEV has a maxium fluorescence emission near 460 nm (when excited at 340 nm, curve a), which is quenched by nucleic acids by 81 percent. (Curve a,b). When different metal ions are added, it is obvious that fluorescence intensity of the systems is quenched in different extent and has the order: $Cu^{2+} > Co^{2+} > Fe^{3+}$. They respectively quenched by 43 percent (curve a, c), 35 percent (curve a, d) and 25 percent (curve a, e). So Cu^{2+} is selected. But different copper salts have different effect on the fluorescence intensity and have the order: $CuCl_2 > CuSO_4 >$ Cu $(NO_3)_2$.

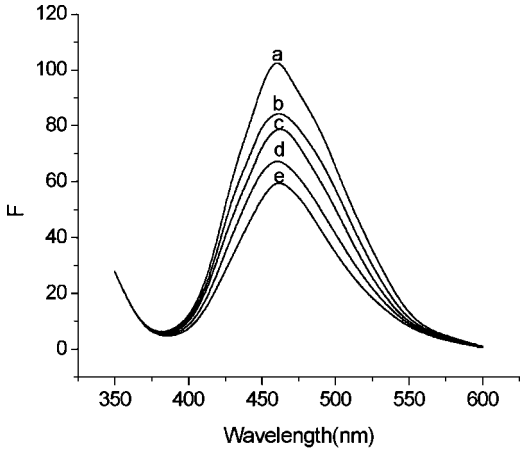

Fig. 2. The selection of metal ion; LEV: 1.95×10^{-6} mol/L; DNA: 1.2×10^{-5} mol/L; Cu^{2+}: 8.0×10^{-7} mol/L; Co^{2+}: 8.0×10^{-7} mol/L; Fe^{3+}: 8.0×10^{-7} mol/L; a: LEV; b: LEV + DNA; c: LEV + DNA + Cu^{2+}; d: LEV + DNA + Co^{2+}; e: LEV + DNA + Fe^{3+}.

Spectral Characteristics of LEV- Cu^{2+}-DNA System

The fluorescence spectra of LEV and its decreased fluorescence intensity by Cu^{2+}, DNA and Cu^{2+} + DNA are shown in Fig. 3. In the system, when 1.0 mL of DNA was added, the fluorescence intensity of LEV was quenched by 23 percent at 460 nm (Fig. 3 curve a, b). In the present of Cu^{2+}, the intensity of fluorescence of LEV can be quenched apparently by 30 percent (Fig. 3 curve a, c). When DNA and Cu^{2+} are added at the same time, the fluorescence intensity of LEV is obviously decreased lowest by 53 percent (Fig. 3 curve a, d). This result might suggest the form of a no-fluorescence ground state ternary complex of LEV-Cu^{2+}-DNA.

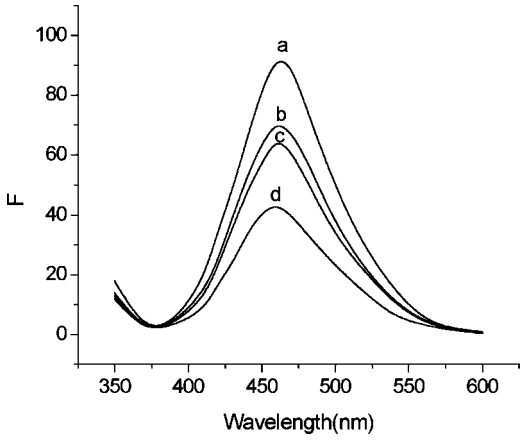

Fig. 3. Fluorescence spectra of LEV-Cu^{2+}-DNA system; LEV: 1.95×10^{-6} mol/L; DNA: 1.2×10^{-5} mol/L; $Cu2^{+}$: 8.0×10^{-7} mol/L; a: LEV; b: LEV + Cu^{2+}; c: LEV + DNA; d: LEV + Cu^{2+} + DNA.

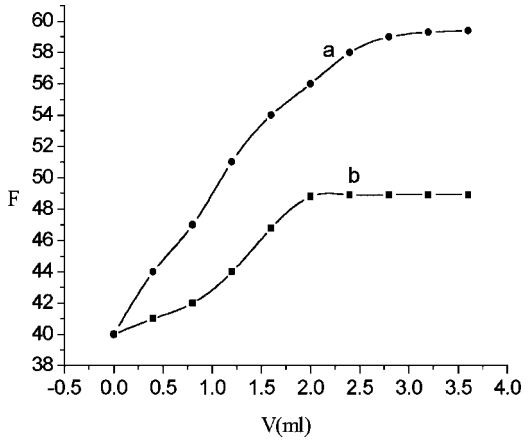

Fig. 4. The effect of ionic strength on the LEV- Cu^{2+}-DNA system; LEV: 1.95×10^{-6} mol/L; DNA: 1.2×10^{-5} mol/L; Cu^{2+}: 8.0×10^{-6} mol/L; a: LEV + Cu^{2+} + DNA + Mg^{2+}; b: LEV + Cu^{2+} + DNA + Na^{+}.

Effect of Ionic Strength

In Fig. 4, the effect of ionic strength controlling by 0.1 mol/L $MgCl_2$ and NaCl solutions are shown. There is little effect on fluorescence intensity of the system in the existence of Na^{+}. However, the fluorescence intensity of the system increases obviously with the increasing of the amount of the Mg^{2+} when Mg^{2+} is added. So it suggested Mg^{2+} competes with LEV-Cu^{2+}, thus leading to an increase of free LEV-Cu^{2+}. The ability of competition of a divalent cation, such as $Mg2^{+}$, is much stronger than that of a monovalent, such as Na^{+}, so just Mg^{2+} can leading to the increase of fluorescence intensity of the system.

Effect of Denatured DNA and Natural DNA

Double-strand DNA was converted into single-strand DNA with the unwinding of its double helix by being incubated at 100°C for 30 min and immediately cooled in ice water for 10 min. As shown in Fig. 5, the interactions of native DNA and denatured DNA with LEV-Cu^{2+} were compared the experimental result showed native DNA can linearly quench the fluorescence intensity of the system of LEV-Cu^{2+} in certain concentration range. The denatured DNA can also quench the fluorescence intensity of LEV-Cu^{2+} system, but its cannot quench the intensity as obviously as the natural DNA. The denatured DNA split into two string-like soft polynucleotide chains from the original rigid double-helix structure, which cause the difference in the fluorescence quenching. The main reason might be associated with the intercalation of LEV-Cu^{2+} into natural DNA base pairs.

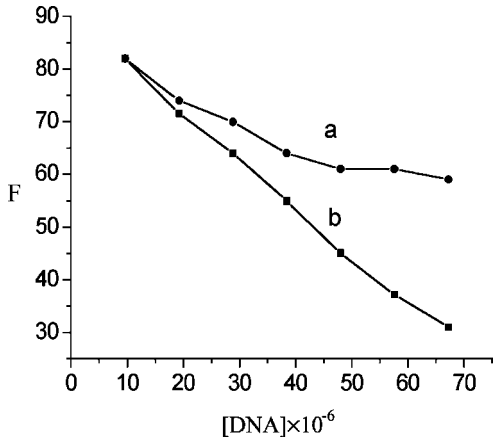

Fig. 5. The effect of denatured DNA and natural DNA on the LEV-Cu^{2+}-DNA system; LEV: 1.95×10^{-6} mol/L; DNA: 1.2×10^{-5} mol/L; Cu^{2+}: 8.0×10^{-6} mol/L; a: LEV + Cu^{2+} + denatured DNA; b: LEV + Cu^{2+} + DNA + natural DNA.

The Affect of Temperatures

The affect of temperatures of the system was studied as follows.

The efficiency of quenching of a fluorophore species by a quencher species follows the Stern-Volmer relationship.

$$F_0/F = 1 + K_{sv}[Q] \tag{1}$$

Where, F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively. Either the collisional quenching of fluorescence or the static quenching of fluorescence can be described by the Stern-Volmer equation. $[Q]$ is the concentration of the quencher. K_{sv} is the Stern-Volmer quenching constant. If a system obeys the Stern-Volmer equation, a plot of F_0/F versus $[Q]$ will give straight line with a slope of K_{sv} and y-axis intercept. For dynamic quenching, K_{sv} increases with increasing temperature. And for static quenching, K_{sv} decreases with increasing solvent's temperature.

Figure 6 is the Stern-Volmer plot of the LEV- Cu^{2+}-DNA system. As shown in Fig. 6, the term, F_0/F, linearly increases with increasing the concentration of quencher. The coefficients are 0.9957 (20°C) 0.9952 (40°C), respectively. At the same time, the quenching efficient of LEV + Cu^{2+} fluorescence by DNA undergoes an intense decrease with increasing temperature, $K_{20°C} = 5600\ \mathrm{L} \cdot\ \mathrm{mol}^{-1}$, $K_{40°C} = 4500\ \mathrm{L \cdot mol^{-1}}$, respectively. It is suggested that the static quenching is attributable to the formation of a non-fluorescence ground state complex between LEV + Cu^{2+} and DNA.

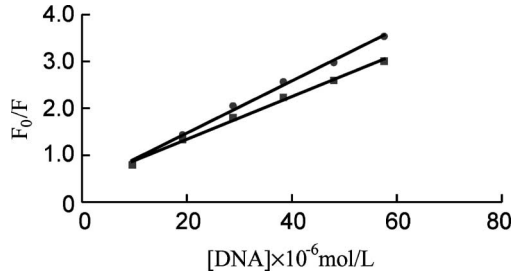

Fig. 6. Fluorescence quenching stern-volmer plots of LEV+ Cu^{2+} with increasing concentration of DNA; LEV: 1.95×10^{-6} mol/L; Cu^{2+}: 8.0×10^{-6} mol/L; circle: 30°C; square: 40°C.

The Composition of the Binary Complex

The composition of the binary complex can be deduced from the following formula [18].

$$M + nL = MLn \tag{2}$$

$$\mathrm{Log}[(F_0 - F)/F] = \log Ka + n \log[M] \tag{3}$$

Where M is the quencher, L is the pharmaceutical molecule with a fluorophore, MLn is binary complex whose resultant constant is Ka, F_0 is the fluorescence of the overall amount of pharmaceutical molecule (bound and unbound), F is the fluorescence of unbound pharmaceutical molecule. A plot of log $[(F_0 - F)/F]$ versus log $[M]$ will give straight line with a slope of n and y-axis intercept logKa.

Keeping the LEV concentration constant and changing the concentration of Cu^{2+} obtain Fig. 7. The data are well fitted to Eq. (3) and the slope is 1.3. The coefficient is 0.9946. The result indicates that the fluoroquibolone antibiotics can form stable 1:1.3 complexes with Cu^{2+}.

The composition of the complex LEV-DNA was also determined by the above method. A 1:0.65 mole ratio of LEV to DNA is conformed by the experimental results. Fig. 8 shows the result. The coefficient is 0.9957. The

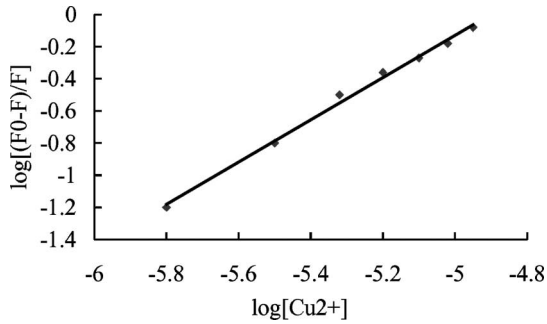

Fig. 7. Estimation of composition of the LEV-Cu^{2+} complex; LEV: 1.95×10^{-6} mol/L.

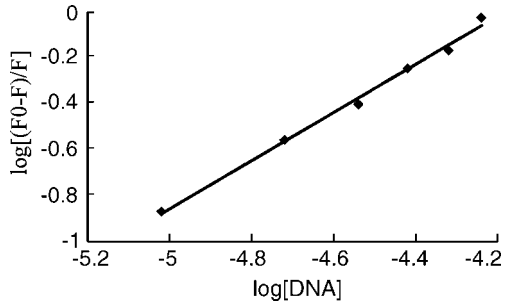

Fig. 8. Estimation of composition of the LEV- DNA complex; LEV: 1.95×10^{-6} mol/L.

mole ratio of LEV to DNA is smaller than that of LEV-Cu^{2+} system. It is suggested that the force between LEV and Cu^{2+} is much stronger than that between LEV and DNA.

The Reaction Mechanism

In this experiment, deduced from the fluorescence, a ground-state binary complex is formed between LEV and Cu^{2+}, and a ground-state ternary complex also can be formed among LEV, Cu^{2+} and DNA. Small molecular integrate with nucleic acids have three binding mechanisms [19]. One is intercalative binding model. Another one is the groove-binding model. The last one is the long-range assembly of the organic dyes on the molecular surface of nucleic acids. If the LEV- Cu^{2+} complex interacts with DNA according to intercalation model, the chromophore would bury itself in the stack of DNA bases, leading to spectral changes caused by the interactions of the complex with the electronic states of the DNA bases. Aromatic $\pi-\pi^*$ states of LEV-Cu^{2+} seem to interact strongly with the electronic states of the DNA bases.

Earlier studies have pointed out that norfloxacin binds to DNA based via hydrogen bonding. The hydrogen bonding donors thus become available for pairing with the acceptors on the drug molecules (carbonyl or fluoro groups). Both C_4 carbonyl group on the quinolone ring and C_3 carboxylic acid group are potential hydrogen-bond acceptors. But it is discovered that the interaction between LEV and DNA is weak without Cu^{2+} in the experiment. The result of experiment indicated a possible interaction mechanism for LEV binding to double-stranded DNA. That is, firstly, LEV forms a binary complex with Cu^{2+}, and then forms a ternary complex with DNA by intercalation binding form, and finally, releases Cu^{2+} to form hydrogen bonds between LEV and DNA. Cu^{2+} plays an intermediary role in the interaction between the LEV and DNA.

CONCLUSIONS

In summary, the fluoroquinolone antibiotics, LEV, can complex with Cu^{2+} and DNA, which gives information concerning the antibiotic-nucleotide interaction. From the experimental results, we can conclude that LEV can form a steady binary complex with LEV-Cu^{2+} can interact with DNA by intercalative binding model, and Cu^{2+} plays an intermediary role. This behavior is of great importance with regard to relevant biological role of fluoroquinolone antibiotics in the human body.

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