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Influence of metal ions on the interaction between gatifloxacin and calf thymus DNA

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ABSTRACT

To study the interaction between gatifloxacin (GT), metal ions (Cu^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}) and calf thymus DNA under condition of physiology pH, UV absorption and fluorescence methods were adopted. Result shows that metal ions and DNA are able to react with GT in ground state. In further research, by studying the influence of metal ions on binding of GT with DNA in metal ions–GT–DNA ternary system, we found that influential mechanism of Mg^{2+} on the binding of GT with DNA may be different from the other three. Mg^{2+} can act as a bridge in the binding of GT's carboxyl/carbonyl with DNA phosphate in certain concentration range; while Cu^{2+}, Cd^{2+}, Co^{2+} can combine directly with GT by reaction between GT carboxyl/carbonyl and DNA base, and enhance the binding ability of GT with DNA. The influence extent and type depend not only on the binding site of DNA with metal ions (phosphate or base), but also the binding ability of which. The stronger the binding ability of metal ions with DNA base is, the larger their promotion to binding of GT with DNA is. The order of metal ions' influential ability on the binding of GT–DNA is identical to the binding ability order of metal ions with DNA base, that is: $Cu^{2+} > Cd^{2+} > Co^{2+} > Mg^{2+}$.

1. Introduction

Quinolones are a group of important synthetic antibacterial agents with characteristics of wide antibiogram and strong antibacterial ability [1]. Former studies indicated that quinolone develops its pharmacological action via specific inhibition to the bacterial gyrase [2]. Although the exact mechanism of this action is still unclear, there is evidence that quinolone interacts directly with DNA by synergying with gyrase [3]. Such interaction undoubtedly contributes to the desired antibacterial activity. However, it can also be responsible, at least in part, for the unwanted toxic effects [4]. Therefore, contributions to deeper insight into the interaction mechanism of these antibiotics with DNA might be important for a better understanding to their therapeutic efficacy, further design amelioration of quinolone drugs and relationship of nucleic acid biomacromolecule structure and function.

Lots of studies on binding of DNA with quinolone have been developed. Shen and coauthors have proposed DNA-drug models which imply hydrogen-bond type interactions between the DNA unpaired bases and quinolone [5]. Palumbo et al., have stressed that Mg^{2+} acts as a bridge between quinolone and DNA phospho, and this complex is stabilized by stacking interactions between the condensed rings of drug and DNA base in a single-stranded region or a distorted B-form in plasmid [6]. Llorente et al. have proposed another model based on the interaction of quinolone with double helix of DNA [7]. In recent studies, an important role of metal ions was proposed again. After studying on the structure and activity of certain quinolones and interaction of their Cu^{2+} complexes on a DNA model, scientists suggested that the formation of quinolone-metal complex is an important step in these reactions [8].

The interaction of quinolones with DNA and function of metal ions in these processes have been extensively studied in the past. The topic is extremely important due to the fact that more and more quinolones are used in clinical practice. But, people's understanding on the binding model of quinolone with DNA and influential mechanism of different metal ions on this process were still limited and unconfirmed.

In this study, the interaction between the 4th generation quinolone-gatifloxacine (GT) and CT–DNA complex were analyzed. Four metal ions which have their order of base-binding/phospho-binding ability like $Cu^{2+} > Cd^{2+} > Co^{2+} > Mg^{2+}$ [9] were selected. With the methods of UV absorbance and fluorescent, we hope to get further information about the interaction between quinolones and DNA, and general rule about influence of metal ions on them.

2. Materials and methods

2.1. Apparatus and materials

The absorption and fluorescence spectra were obtained by using UV-2450 spectrophotometer and RF-5301PC fluorescence spectrophotometer (Shimdazu, Japan). GT (HaoSeng Co Ltd., JiangSu,

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China, content >99.97%) was kept at 4 °C and dark in solid state, prepared into storage solution (concentration is 1×10^{-3} M) with NaCl (concentration is 0.02 M) prior to use. CT–DNA (HuaMei Biology Engineering Company Beijing Branch Office, purity, $A_{260}/A_{280} = 1.8$) solution was prepared by dissolving the DNA in 0.02 M NaCl solutions. DNA concentrations were expressed as DNA phosphate with a molar extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm [10]. $CuCl_2$, $CdCl_2$, $CoCl_2$, $MgCl_2$ and other reagents were all analytical reagents.

2.2. Methods

The solution being measured were adjusted and kept at pH 7.0 by tris–HCl buffer (0.01 M) at ambient temperature, and UV absorbance and fluorescent spectrum were measured, respectively. In fluorescent model, both excitation and emission bandwidths were set at 15 nm, excitation wavelength at 340 nm, and fluorescence value at emission wavelength of 450 nm were recorded.

GT–DNA, GT–Cu^{2+}, GT–Cd^{2+}, GT–Co^{2+} and GT–Mg^{2+} binary systems were formed by titrating GT solution with DNA, $CuCl_2$, $CoCl_2$, $CdCl_2$ and $MgCl_2$ separately. The concentration of GT was maintained at 1×10^{-5} M in both UV absorption spectra and fluorescence spectra determination. GT–DNA–metal ions ternary systems were formed by titrating GT–DNA binary system with $CuCl_2$, $CoCl_2$, $CdCl_2$ and $MgCl_2$. During the titration operations, DNA, $CuCl_2$, $CoCl_2$, $CdCl_2$ and $MgCl_2$ stock solutions were added with microliter quantities [11].

3. Results and discussion

3.1. Binary binding of GT with Cu^{2+}, Co^{2+}, Cd^{2+} and Mg^{2+}

After titrating GT solution with Cu^{2+}, Co^{2+} and Cd^{2+} separately, the fluorescence of the solution quenched obviously (Fig. 1), while with Mg^{2+} the fluorescence of the solution enhanced. The reason of fluorescence quench on GT may be complex reactions occurring between metal ions and oxygen atoms of GT 3-carboxyl group and 4-carbonyl group [12], thus the reduction of GT molecular conjugacy leads to its fluorescence quench.

Binding constant K of reaction between GT and four metal ions can be calculated by following two formulas [13]:

$$\lg\left[\frac{F_0 - F}{F}\right] = \lg K + n \lg[Q] \quad \text{(Fluorescence quench)} \tag{1}$$

In Eq. (1), F is the relative fluorescence intensity (RFI) of free drug in the system; F_0 is the RFI of total drug; $[Q]$ is equilibrium concentration of quencher; K is binding constant of drug with quencher; n is binding site number of quencher with drug (see Table 1).

$$(F_{cm} - F_c)^{-1} = K_a^{-1} \cdot K_i^{-1} Q_{cm}^{-1} [C]_0^{-1} \cdot [M]_0^{-1} + K_i^{-1} Q_{cm}^{-1} [C]_0^{-1}$$
$$= B \cdot [M]_0^{-1} + A \quad \text{(Fluorescence enhancement)} \tag{2}$$

In Eq. (2), $(F_{cm} - F_c)$ is RFI change of drug before and after adding sensibilizer; $[M]_0$ is the concentration of sensibilizer; $[C]_0$ is initial concentration of drug; Q_{cm} is quantum yield of drug-sensibilizer complexes; K_i is the instrument constant. Construct a curve by $1/(F_{cm} - F_c)$ versus $1/[M]_0$, binding constant of drug with sensibilizer (K) can be obtained from curve. If the result is of good data relativity, it means 1:1 complexes are formed (see Table 1).

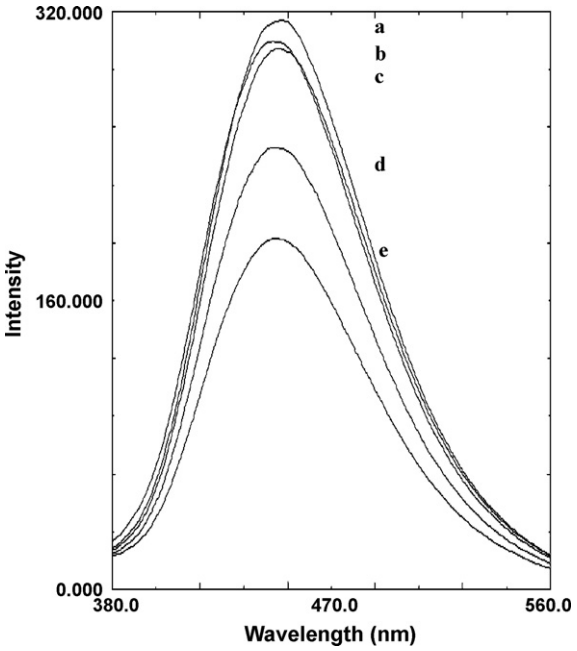

Fig. 1. Fluorescence changes that occur when GT is titrated with metal ions: pH 7.0, C_{NaCl} (M): 0.02; λ_{ex} = 340 nm. C_{GT} (M): 1.0×10^{-5}; C_{Mg} (M): 3.33×10^{-3}; C_{Cd} (M): 1.33×10^{-3}; C_{Cu} (M): 2.0×10^{-5}; C_{Co} (M): 3.33×10^{-5}. a: GT + Mg, b: GT, c: GT + Cd, d: GT + Cu, e: GT + Co.

Fluorescence quench can be distinguished between dynamic and static quench. The general method of distinguishing whether dynamic or static is UV absorption spectra determination [14]. In the process of static quench, the forming of ground state complexes often leads to the absorption spectra change of absorption substance [15]. To confirm the occurrence of ground state complex reactions of Cu^{2+}, Cd^{2+}, Co^{2+} with GT, we studied UV absorption spectra of the system (Fig. 2).

According to Fig. 2, Cu^{2+}, Cd^{2+}, Co^{2+} and Mg^{2+} can cause red shift and reduction of UV absorption on GT. There are iso-absorptive points which occur at 285 nm and 322 nm in spectra of Cu^{2+}–GT binary system (Fig. 2A), 283 nm and 315 nm in Cd^{2+}–GT system (Fig. 2B), 287 nm and 318 nm in Co^{2+}–GT system (Fig. 2C), 280 nm and 317 nm in Mg^{2+}–GT system (Fig. 2D). This proves that all the four metal ions can react with GT and form stable ground state complexes with determinate complex ratio.

3.2. Binary binding of GT with DNA

When DNA was added into GT solution, system fluorescence quenches (Fig. 3). The relationship of $F_0 - F$ (fluorescence intensity change of DNA added system) with Q (concentration of DNA) can be explained by formula (1) [13], and the binding constant of GT with DNA can also be calculated: $K = 506\ M^{-1}$ ($r = 0.9980$, data number = 7), $n = 1$. UV absorption of GT–DNA binary system was also

Table 1
Binding constants of GT with Cu^{2+}, Cd^{2+}, Co^{2+} and Mg^{2+}.

Chemicals	Data number	r (correlation coefficient)	n (n^*)	K (binding constant, M^{-1})
GT–Cu	7	0.9961	1	6280
GT–Cd	7	0.9855	1	21
GT–Co	7	0.9999	1	9128
GT–Mg	7	0.9910	1	7.9

K: represent binding constant; n: represent binding site number; n^*: represent complex ratio.