Asian Journal of Chemistry; Vol. 24, No. 5 (2012), 2061-2064

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Studies of Interaction of Two Common Drugs and DNA in the Presence of Neutral Red by Fluorescence Spectroscopy and RLS Spectroscopy Method

MIAOMIAO YAN and GUANGCHENG WEI*

Department of Pharmacy Science, Binzhou Medical College, Yantai 264003, P.R. China

*Corresponding author: E-mail: yanmm81@163.com

(*Received*: 23 April 2011; *Accepted*: 12 December 2011) AJC-10828

Detailed analyses of the interaction of drugs (cefoperazone and acheomycin) with DNA by fluorescence, resonance light scattering spectroscopic techniques are carried out in this work. All the evidences show that the cefoperazone and acheomycin both can affect the fluorescence of neutral red-DNA system. Cefoperazone and acheomycin obviously can quench the resonance light scattering spectrum of neutral red-DNA system, it indicates that the drugs can combing with DNA and damage the long distance assembly of neutral red in the DNA surface. The binding mode of acheomycin and DNA is complicated than cefoperazone.

Key Words: DNA, Cefoperazone, Acheomycin, Neutral red, Fluorescence spectroscopy, RLS spectroscopy.

INTRODUCTION

In recent years, the mechanism of the binding of small molecules, such as drug[1-6], organic dyes[7-11] to DNA has been an active area. It is a subject that exists at the interface of pharmaceutical chemistry and biology. The intracellular target for the majority of anticancer and antibiotic drugs is DNA[12]. So, the study on the interaction of drug and DNA plays a key role in pharmacology and it is of great significance for designing and synthesizing the new drugs targeted to DNA and their effectiveness depends on the mode and affinity of the binding. Cefoperazone and acheomycin (Fig. 1) are common antibiotic drug, the interest is aroused to study the relationship of drugs (cefoperazone and acheomycin) with DNA. The fluorescence quantum yield of DNA is about 10^{-4} to 10^{-5} at room temperature[13,14] and the intrinsic fluorescence from DNA is of little practical usefulness. The utility of fluorescence probes can obtain the information of the structure and quantitative of DNA. The probes for nucleic acids reported include organic dyes, such as neutral red (NR) (Fig. 2), ethidium bromide (EB)[15-17], bisimidazole (Hoechst 33258)[18], metal complex[19] and photosensitive agent[20]. Neutral red was used to probe the interaction of drugs (cefoperazone and acheomycin) with DNA in this work. DNA binding with drugs (cefoperazone and acheomycin) cannot be found by fluorescence method without the probe neutral red, but it is clearly seen that the fluorescence and resonance light scattering of DNA-neutral red system can be quenched by drugs. The fluorescence and resonance light scattering change of DNA-neutral red in the presence of drugs suggests that there is a strong interaction between the drugs and DNA.

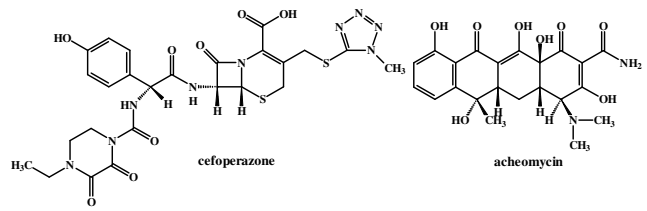

Fig. 1. Structure of cefoperazone and acheomycin

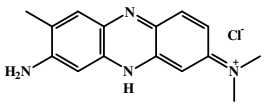

Fig. 2. Structure of neutral red

EXPERIMENTAL

A Perkin Elmer LS55 fluorescence spectrometer (United Kingdom) with a quartz cell of 1 cm path length is used to measure the fluorescence and resonance light scattering spectra; Elix10 + MilliQ pure water system (USA); protein-DNA analysis instrument.

Deoxyribonucleic acid from the liver of white mouse is purchased from Sigma Chem. Co. and its stock solution is prepared by dissolving appropriate solid DNA into pure water overnight and its concentration is determined by absorption spectrometry, using the molar absorptivity $\varepsilon_{260} = 6600$ mol^{-1}

cm^{-1}. Stock solution of 3.0×10^{-5} mol L^{-1} DNA and 1.0×10^{-6} mol L^{-1} should be stored at 4 ºC in the dark for about a week only. The ratio of the absorbance at 260 nm to that of 280 nm is checked with protein-DNA analysis instrument to monitor the purity of DNA. The solution gave a ratio of > 1.8 at A_{260}/A_{280}, indicating that DNA is sufficiently free from protein[21], neutral red is obtained from Chemical Reagent Co. of Tianjin and the concentration of stock solution of neutral red is 1×10^{-6} mol L^{-1}. Cefoperazone and acheomycin from China Drug Biological Products Qualifying Institute is prepared into 1.0×10^{-6} mol L^{-1} water stock solutions. DNA in this work is double stranded DNA unless it is especially noted clearly. All reagents are of analytical reagent grade and the pure water is used all along. The fluorescence spectra and resonance light scattering of a fixed amount of DNA in pH 7.25 *tris*-HCl buffer are measured in the absence and in the presence of neutral red.

Neutral red binding with DNA: The fluorescence spectra of a fixed amount of DNA in pH 7.25 *tris*-HCl buffer are measured in the absence and in the presence of neutral red. The excitation wavelength and emission wavelength are at 310 and 624 nm. The slits width of the excitation is 10 nm and emission is 15 nm. The fluorescence spectrum is scanned from 593 to 648 nm.

The resonance light scattering spectra of a fixed amount of DNA in pH 7.25 *tris*-HCl buffer are measured in absence and in presence of neutral red. The excitation wavelength and emission wavelength are all 310 nm. The slits width of the excitation is 10nm and emission is15 nm. The resonance light scattering spectrum is scanned from 270 to 600 nm.

Drugs binding with DNA: The fluorescence and resonance light scattering spectra of a series of solutions with various volume of cefoperazone are measured in DNA-neutral red complex system. Before measurements, the tubes should be shaken up and placed into the thermostat water bath for 5 min. Then the assay solutions are transferred into the quartz cell with a micro-stirrer and the fluorescence and resonance light scattering measurements are performed.

RESULTS AND DISCUSSION

Fluorescence spectra: The nature of the interaction of neutral red with nucleic acids was studied by many research reports[22-24], the interaction fluorescence spectra DNA and neutral red are showed in Fig. 3.

Fig. 3 shows that neutral red has very strong fluorescence in water solution (λex = 310 nm,λem = 593.7-648.4 nm). The curve 1 is the fluorescence intensity of neutral red without DNA, with the DNA added in the neutral red solution, the change of fluorescence intensity is obvious. When R (the mole ratio of DNA to neutral red) = 0.3 (2, 3, 4 in Fig. 3), the fluorescence intensity has enhancement and R≈ 0.1125 (the curve 2 in Fig. 3), the fluorescence intensity is strongest. When R > 0.3, the fluorescence intensity decrease (the curve 5, 6, 7, 8 in Fig. 3).

The reason may be:

(1) The nucleic acid has phosphorus acid radical, as a result negative charge existence. Neutral red solution has the positive charge, when the DNA was added to the neutral red solution, binding occurs between DNA and neutral red by static electricity function linkage, the binding constant is big. It is equal that the small dye molecular piles up on the nucleic acid molecular. It causes the probability, which the excited state of neutral red occurs outer shifts to reduce, therefore fluorescence intensity enhancement.

(2) The phenazine plane of neutral red intercalates into the DNA base pairs, which causes the probability of excited state of neutral red and the solvent collision reduces, as a result the probability of outer shift reduces, so fluorescence intensity enhancement.

(3) It is between both of above referred, the groove interaction, which causes too the probability which the excited state of neutral red occurs outer shifts to reduce, therefore fluorescence intensity enhancement[25].

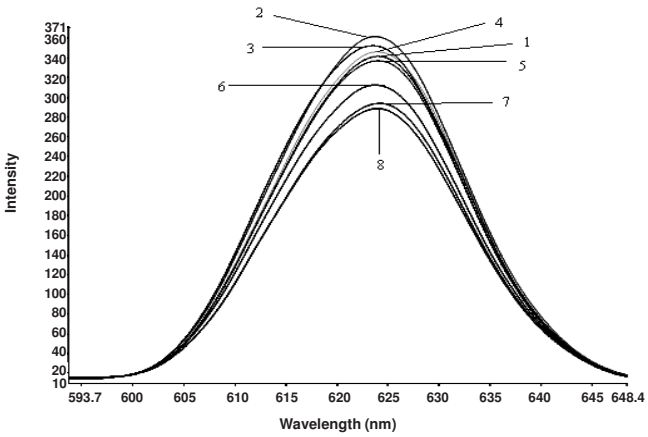

Fig. 3. Fluorescence emission spectra of NR-DNA system V(NR) = 2 mL, c(DNA) = 3.0×10^{-5} mol L^{-1}, corresponding to 1→8 (DNA volume added to NR solution): 0, 7.5, 15, 20, 30, 40, 50, 60 μL. (λex = 310 nm; Ex slit =10 nm; Em slit =15nm)

The reason of fluorescence decrease is that when the reaction of neutral red with DNA finished, the excessive DNA causes the probability of outer shift enhancement, so the fluorescence intensity decrease.

Drugs are added to the neutral red-DNA system (neutral red 2 mL, DNA 15 μL), Fig. 4 are the relative fluorescence intensity of the NR-DNA system as a function of the drug volume.

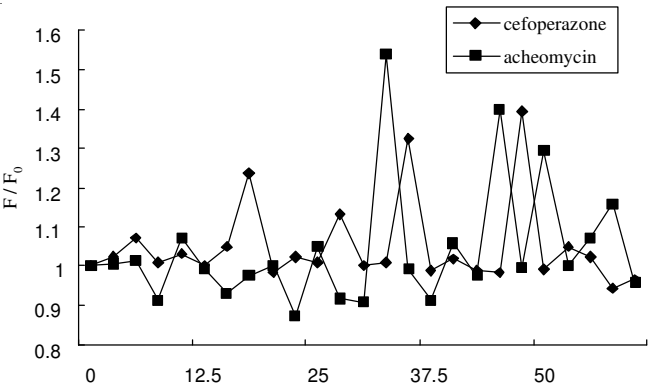

Fig. 4. Relative fluorescence intensity of the neutral red-DNA system as a function of the drug volume, c(drug) = 10^{-6} mol L

Fig. 4 shows that the interaction of drug and DNA is very complex, in general, with the drug volume rising, the extent

of neutral red-DNA fluorescence intensity rising is bigger than the intensity of decreasing, the acheomycin is more obvious than the cefoperazone. Jiang *et al.*[22] reported that the neutral red may also combine with DNA ribose or phosphate ingredient by the electrostatic attraction way under neutral condition, it can change the micro environment, for example the water molecular reduction around the DNA and causes spiral structure of DNA more stable. Wang *et al.*[23] reported that the binding mode of neutral red and DNA can transform between intercalation interaction and electrostatic attraction under the different condition. According to the results of experiment, it is concluded that the binding mode may simultaneously be several basic functions when the reaction of drugs and DNA occur can be obtained and the binding mode of acheomycin to DNA is more complex than the cefoperazone to DNA. The interaction of acheomycin and DNA is strong than the cefoperazone.

Resonance light scattering spectra: The nucleic acid molecular has phosphorus acid radical, as a result the negative charge existence. The small organic dye molecular with positive charge can bind with DNA by the electrostatic attraction and the binding constant is very big. It is same that the small dye molecular piles up on the surface of DNA and cause the enhancement of resonance light scattering[26]. The enhancement of resonance light scattering is caused by the excitation of electron isolated in the super spiral structure of DNA. The resonance light scattering signal of DNA itself is very weak, neutral red has the resonance light scattering signal in the range of 380-400 nm and the resonance light scattering signal will be enhancement when DNA is added to the neutral red solution. The biggest signal intensity is in 390 nm and the variety is very sensitive.

Fig. 5 is the resonance light scattering spectra of neutral red-DNA system. It shows that the resonance light scattering intensity enhance when DNA is added to the neutral red solution.

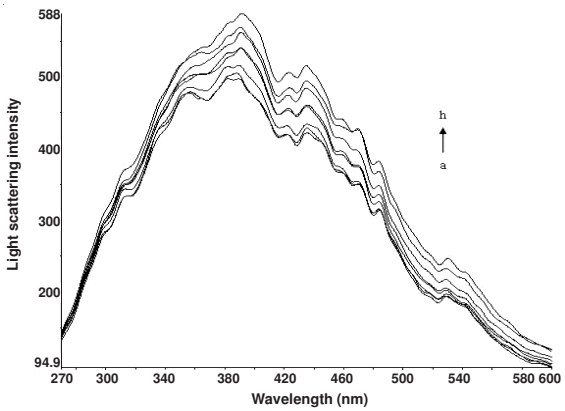

Fig. 5. Resonance light scattering spectra of neutral red-DNA system c(neutral red) = 1.0×10^{-6} mol L^{-1}, V(neutral red) = 2 mL; c(DNA) = 1.0×10^{-6} mol L^{-1}, corresponding to a→h (DNA volume added to neutral red solution) 0, 50, 100, 150, 200, 250, 300, 500 μL λex = 310 nm; Ex slit =10 nm; Em slit =15 nm)

The literature indicates that the reaction of neutral red and DNA involves in H accumulation. This H accumulation can cause the high resonance scattering signal and the signal may be quenched by ethidium bromide, namely intercalation function affect the H accumulation reaction, so the conclusion that H accumulation reaction does not involve in the reaction of intercalation and grooving can be obtained. The resonance light scattering enhancement function of DNA to neutral red is because the long distance assembly of neutral red molecular on DNA molecular surface[27,28]. The DNA induces neutral red molecular accumulation on DNA surface, the action mainly depends on the static electricity function of neutral red and phosphodiesters in DNA structure, possibly also involves in the hydrophobic interaction of the intermolecular. The superficial accumulation is broken when the drugs are added to neutral red-DNA system, the reaction of the drug and DNA causes the depolymerizing of neutral red on the DNA surface and the neutral red molecular dissociate from the neutral red-DNA system. It causes the particle size of neutral red-DNA union small and the intensity decrease of resonance light scattering. The other possibility may be that the interaction of drugs molecular and DNA obviously change the structure of DNA double strand and affect the interaction of neutral red and DNA.

Figs. 6 and 7 are the resonance light scattering spectra of neutral red-DNA-drugs system, the results shows that the change of resonance light scattering is very obvious when drugs are added to the neutral red-DNA system. The drugs can obviously quench the resonance light scattering of neutral red-DNA system and the intensity of resonance light scattering decrease rapidly with the volume of drugs in 380 nm. The difference of resonance light scattering change, which is caused by two drugs is obvious and the rule of the intensity decrease which is caused by cefoperazone is simple, however the rule of the intensity decrease, which is caused by acheomycin is complex. It indicates that the binding mode of cefoperazone and DNA may be single and it may is static electricity attraction mode or hydrophobic interaction mode; however the binding mode of acheomycin to DNA is complicated, so it is difficult to confirm the binding mode and it perhaps has several modes. Regardless of which binding way, they can all affect the long distance assembly of neutral red obviously in the DNA surface.

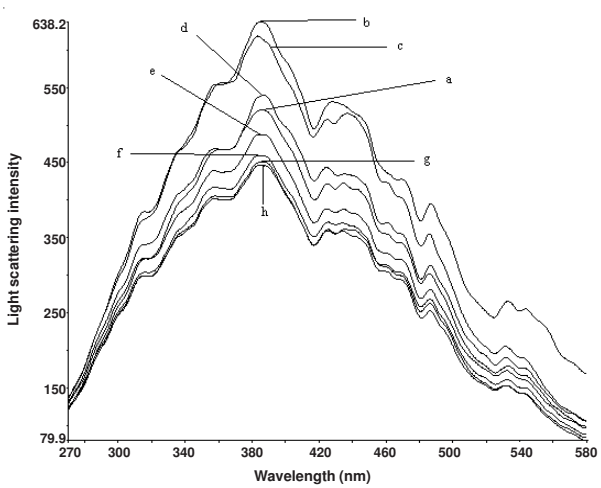

Fig. 6. Resonance light scattering spectra of DNA-neutral red-cefoperazone system c(neutral red) = 1×10^{-6} mol L^{-1}, V(neutral red) = 2 mL; c(DNA) = 1×10^{-6} mol L^{-1}, V(DNA) = 500 μL; c(cefoperazone) = 1×10^{-6} mol L^{-1}, corresponding to b → h (cefoperazone volume added to neutral red-DNA system) 0, 100, 200, 300, 400, 500, 600 μL a: The resonance light scattering spectra of neutral red 2 mL; b: The resonance light scattering spectra of neutral red-DNA system (500 μL DNA added to 2 mL neutral red solution) λex = 310 nm; Ex slit =10 nm; Em slit =15nm)

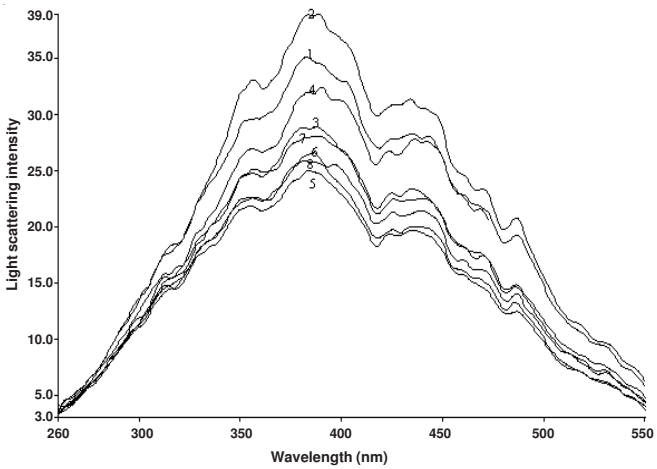

Fig. 7. The RLS spectra of DNA-neutral red-acheomycin system; c(neutral red) = 1×10^{-6} mol L^{-1}, V(neutral red) = 2 mL; c(DNA) = 1×10^{-6} mol L^{-1}, V(DNA) = 500 µL; c(acheomycin) = 1×10^{-6} mol L^{-1}, corresponding to b→h (acheomycin volume added to neutral red-DNA system) 0, 100, 200, 300, 400, 500, 600 µL; (a) The RLS spectra of neutral red 2 mL; (b) The RLS spectra of neutral red-DNA system (500 µL DNA added to 2 mL neutral red solution); (λex = 310 nm ; Ex slit =10 nm; Em slit = 15 nm; 1 % attenuation)

Conclusion

Detailed analyses of the interaction of drugs (cefoperazone and acheomycin) with DNA by fluorescence, resonance light scattering spectroscopic techniques are carried out in this work. All the evidences show that cefoperazone and acheomycin both can affect the fluorescence of neutral red-DNA system, the influence of acheomycin is more obvious than acheomycin. Cefoperazone and acheomycin obviously can quench the resonance light scattering spectrum of neutral red-DNA system, it indicates that the drugs can combing with DNA and damage the long distance assembly of neutral red in the DNA surface. The results show that the quenching mode of acheomycin is more complicated than cefoperazone. It indicates that the binding mode of acheomycin is complicated than cefoperazone. So the interaction of acheomycin and DNA is more complicated and obvious than the cefoperazone. The study of drugs(cefoperazone and acheomycin) and DNA will provide necessary information on the mechanism of anticancer drugs binding with DNA and they will benefit the designing of new drugs.

ACKNOWLEDGEMENTS

Financial support by a Project of Shandong Province Higher Educational Science and Technology Program (Grant No. J09LC66) and by Shandong Provincial Natural Science Foundation, China (Grant Nos. ZR2010BL026 and ZR2011BL026) is gratefully acknowledged.

REFERENCES

1. P. Drevenšek, I. Turel and N.P. Ulrih, *J. Inorg. Biochem.*, **96**, 407 (2003).
2. V. Maleev, M. Semenov, E. Kruglova, T. Bolbulh, A. Gasan, E. Bereznyak and A. Shestopalova, *J. Mol. Struct.*, **645**, 145 (2003).
3. M.A. Khan and J. Musarrat, *Int. J. Biol. Macromol.*, **33**, 49 (2003).
4. Y.L. Zhou and Y.Z. Li, *Biophys. Chem.*, **107**, 273 (2004).
5. C. Tu, X. Wu, Q. Liu, X. Wang, Q. Xu and Z. Guo, *Inorg. Chim. Acta,* **357**, 95 (2004).
6. Y. Ni, D. Lin and S. Kokot, *Talanta,* **65**, 1295 (2005).
7. M.B. Lyles and I.L. Cameron, *Biophys. Chem.*, **96**, 53 (2002).
8. L. Vergani, G. Mascetti, P. Gavazzo and C. Nicolini, *Thermochim. Acta,* **294**, 193 (1997).
9. Y. Cao, X.W. He, Z. Cao and L. Peng, *Talanta,* **49**, 377 (1999).
10. H.S. Rye, J.M. Dabora, M.A. Quesada, R.A. Mathies and A.N. Glazer, *Anal. Biochem.*, **208**, 144 (1993).
11. M.V. Lancker and L.C. Gheyssens, *Anal. Lett.*, **19**, 615 (1986).
12. L.H. Hurley and F.L. Boyd, *Trends Pharm. Sci.*, **9**, 402 (1988).
13. P. Vigny and A. Favre, *Photochem. Photobiol.*, **20**, 345 (1974).
14. J.P. Morgan and M. Daniels, *Photochem. Photobiol.*, **31**, 101 (1980).
15. W. Fuller and M.J. Warning, *Ber. Bunsenges. Phys. Chem.*, **68**, 805 (1964).
16. C.G. Reinhardt and T.R. Krugh, *Biochemistry,* **17**, 4845 (1978).
17. J.B. Lepecq, C.C. Paoletli, *Anal. Biochem.*, **17**, 344 (1996).
18. C. Labara and K. Paigen, *Anal. Biochem.*, **102**, 344 (1996).
19. C.Z. Huang, K.A. Li and S.Y. Tong, *Anal. Lett.*, **30**, 1305 (1997).
20. W.Y. Li, X.Q. Guo, J.G. Xu, Q.Z. Zhu and Y.B. Zhao, *Anal. Chim. Acta,* **340**, 291 (1997).
21. J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).
22. X. Jiang, L. Shang, Z.-X Wang and S.-J. Dong, *Biophys. Chem.*, **118**, **42** (2005).
23. Z.-X. Wang, Z.-L. Zhang, D.-J. Liu and S.-J. Dong, *Spectrochim. Acta A,* **59**, 949 (2003).
24. Z. Jia, J.-H. Yang, X. Wu, C.-X. Sun, S.-F. Liu, F. Wang and Z.-S. Zhao, *Spectrochim. Acta A,* **64**, 555 (2005).
25. S.Y. Bi, H.Q. Zhang, C.Y. Qiao, Y. Sun, C.M. Liu, *Spectrochim. Acta A*, **69**, 123 (2008).
26. C.-Z. Huang, K.-A. Li and S.-Y. Tong, *Bull. Chem. Soc. Jpn.*, **70**, 1843 (1997).
27. H.-Y. Xiang, X.-M. Chen, S.-Q. Li, S. Xia and A.-X. Liu, *Spectrsc. Spectr. Anal.*, **21**, 822 (2001).
28. C.-Y. Yang, Y. Liu, F. Zeng, J. Li, Q.-G. Li and L.-W. Li, *Acta Chim. Sin.*, **5**, 18 (2007).