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Evaluation of the Potential Toxicity of Anthraquinone Derivatives in Chinese Herbal Medicines by the Resonance Light Scattering Spectrum

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In this paper, resonance light scattering method has been used to evaluate potential toxic mechanism of physcion, chrysophanol and rhein interaction with DNA, which are based on the interactions with DNA and compared with ethidium bromide (EB), adriamycin and mitoxantrone. And then, the saturation value binding with DNA, which is calculated by the resonance light scattering results, is first put forward as the evaluation index to evaluate the ability of intercalating into DNA and the potential toxicity of anthraquinone molecule. The greater the saturation value is, the stronger the ability of intercalating into DNA and the potential toxicity of anthraquinone molecule are. The results showed that the saturation value of physcion, chrysophanol, rhein, mitoxantrone, adriamycin or ethidium bromide interacting with DNA is 0.15, 0.53, 0.66, 3.31, 10.58, 14.70, respectively. From these results, it can be speculated that physcion, chrysophanol or rhein shows capacity of intercalating into DNA and toxicity, but they are much weaker than those of mitoxantrone, adriamycin and ethidium bromide. This study provides a fast, easy operation and low cost biological detection method for evaluating the potential toxicity of some herbal active molecules.

Key Words: Resonance light scattering, Physcion, Chrysophanol, Rhein, Saturation value, Potential toxicity.

INTRODUCTION

All herb or herbal medicines have been erroneously considered to be gentle, non-toxic and even harmless for a long time in the minds of some people because of their natural origin. However, it is well-known that the consumption of herbal medicine is capable of producing prominent adverse health effects. Due to increased morbidity and mortality, poisonings associated with the use of herbs have raised universal attention in the last few years[1]. Usually, herb or herbal medicines are complex mixtures, containing hundreds of chemically different constituents but only a few, if not one, compounds are responsible for the beneficial and/or hazardous effects[2]. Furthermore, the biologically active compounds form just a minute part of herbs being diluted with a large amount of nucleic acids, proteins, amino acids, lipids, carbohydrates, vitamins, *etc*., which, in some cases, also contribute to the pharmaceutical or toxic effects and they make the potential toxic evaluation of herb or herbal medicines, the quality control of crude drugs and their medical preparations extremely difficult. Although many other pharmacological or toxicity testing, for example, DNA comet assay, cell detection experiments, animal experiments, clinical experiments[3-5] can be used for the evaluation of the potential toxicity of Chinese herbal medicine. These methods are usually cumbersome and longer operating, especially these methods can not make a rapid assessment on efficacy or toxicity of active ingredients affected by the other components. The anthraquinone derivatives are the major active ingredients of many medical herbs and Chinese herbal medicines in the Chinese Pharmacopoeia. However, some of them showed a strong adverse effect and toxic effect[6-10]. As the resonance light scattering (RLS) spectra is a newly developed and sensitive technique for studying the interaction between the organic small flat molecules with DNA[11] and useful for biochemical and pharmaceutical analysis[12], this paper intends to study the interactions between Chinese herbal anthraquinone derivatives and DNA and then build up a novel method for rapidly evaluating the potential toxicity of anthraquinone derivatives by the scattering resonance spectroscopy and the saturation values binding with DNA.

EXPERIMENTAL

A Shimadzu RF-5301PC fluorophotometer (Kyoto, Japan) with a quartz cell of 1 cm path length is used to measure the resonance light scattering.

Natural double DNA used in this study includes herring sperm DNA (fs DNA, sigma). The stock solutions were prepared by dissolving the solid DNA in doubly distilled water with occasionally gentle shaking and stored at 4 ºC. The

concentration of DNA solution was determined by UV absorption at 260 nm using the molar absorption coefficient ε_{260} = 6600 mol^{-1} cm^{-1}. Concentration of DNA in the stock solution was 3.78×10^{-4} mol L^{-1} in the experiment. Dilute to 3.78×10^{-5} mol L^{-1} when it is used.

The stock solution of 1.0×10^{-4} mol L^{-1} physcion, chrysophanol and rhein (physcion, chrysophanol, rhein, National institute for control of pharmaceutical and biological products) was prepared by ethanol solutions. Dilute to1.00×10^{-5} mol L^{-1} when they are used.

The stock solution of 1.0×10^{-4} mol L^{-1} ethidium bromide (EB), adriamycin and mitoxantrone (EB, adriamycin, mitoxantrone, National institute for control of pharmaceutical and biological products) was prepared by dissolving in distilled water. A Britton-Robinson buffer (pH 7.40) was used to control the pH of the reaction system. All reagents were of analytical reagent grade and doubly distilled water was used throughout the experiments.

Procedures: The resonance light scattering spectrum of ethidium bromide, adriamycin, mitoxantrone, physcion, chrysophanol or rhein with DNA. Put 0.1 mL working solution of ethidium bromide, adriamycin, mitoxantrone interacting, physcion, chrysophanol, or rhein and 1 mL of buffer solution into a 10 mL volumetric flask, vortex and then add appropriate DNA solution. The mixture was diluted to 5 mL scale mark by using doubly distilled water and mixed thoroughly. The resonance light scattering spectrum was obtained by scanning simultaneously the excitation and emission monochromators of the RF-5301PC spectrofluorometer from 220-700 nm. The extent of light-scattering was measured at the maximum wavelength with slit width at 15.0 nm for the excitation and emission.

RESULTS AND DISCUSSION

Resonance light scattering spectrum of ethidium bromide, adriamycin and mitoxantrone interacting with DNA: Ethidium bromide is a kind of small molecules with planar structures. Ethidium bromide is recognized as a kind of DNA intercalator. It can parallel intercalate into the base pairs of double-helical DNA and then change the DNA molecule configurations. Therefore, it has been shown to inhibit replication of DNA and it is carcinogenic[13]. Adriamycin is anthracycline antibiotics and it can intercalate into DNA. Therefore, it inhibits DNA and RNA biosynthesis dependency on DNA. Adriamycin is a potent and broad-spectrum antineoplastic agent that plays a major role in cancer chemotherapy. Unfortunately, its use has been hampered by conventional toxicities and cardiotoxicity manifested by congestive cardiomyopathy. It causes nausea, vomiting, alopecia and hematopoietic suppression[14]. Mitoxantrone is a synthetic anticancer agent that exhibits broad antitumor activity and it has been used effectively against breast cancer, acute leukaemia and malignant lymphomas with minimal side-effect. In contrast to the clinically useful anthracyclines daunorubicin and doxorubicin, mitoxantrone appears to exhibit significantly lower cardiotoxcity[15].

The interaction procedure of ethidium bromide, adriamycin or mitoxantrone with fs-DNA in pH 7.4 BR was characterized by the resonance light scattering spectrum. It is shown that the resonance light scattering signal of DNA itself is weak and EB-DNA interaction results in strong enhanced resonance light scattering signals characterized by four peaks at 248, 360, 470 and 580 nm, respectively (Fig. 1). Adriamycin-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 290, 468 and 545 nm (Fig. 2). Mitoxantrone-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 290, 468 and 570 nm (Fig. 3). Resonance light scattering of liquid particles show that illuminant, molecular absorption and resonance scattering effect of associating particles are three main factors of synchronous scattering peaks[16]. Taking ultraviolet absorption and sensitive peak into consideration, present study selected the resonance scattering peak of ethidium bromide at 580 nm to investigate and the resonance scattering peak of adriamycin or mitoxantron at 468 nm. This resonance light scattering enhancing phenomenon shows that ethidium bromide, adriamycin or mitoxantronmay combine with DNA to form a compound, resulting in the formation of super-helical structure of nucleic acid. The resonance light scattering show that ethidium bromide, adriamycin and mitoxantron intercalate DNA.

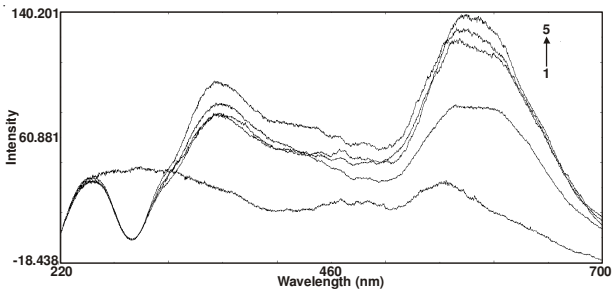

Fig. 1. Resonance light scatting spectra of DNA and ethidium bromide. C_{EB} 2-5: 2×10^{-5} mol L^{-1}, C_{DNA} 1-5: 1.134×10^{-7}, 0, 7.56×10^{-7}, 1.058×10^{-6}, 1.361×10^{-6} mol $\cdot L^{-1}$

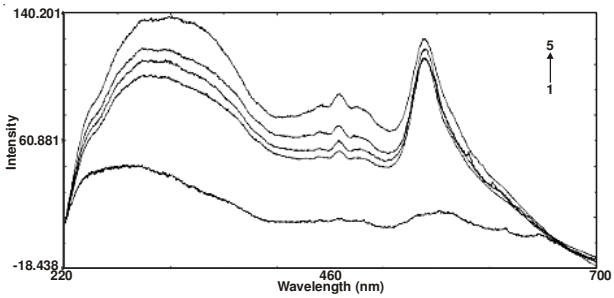

Fig. 2. Resonance light scatting spectra of DNA and adriamycin $C_{adriamycin}$ 2-5: 4×10^{-5} mol L^{-1}, C_{DNA} 1-5: 3.024×10^{-7}, 0, 2.268×10^{-7}, 3.024×10^{-6}, 3.78×10^{-6} mol L^{-1}

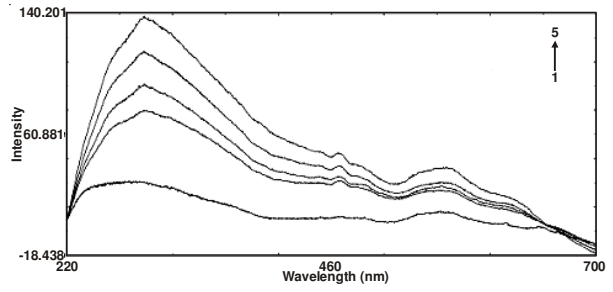

Fig. 3. Resonance light scatting spectra of DNA and mitoxantrone. $C_{mitoxantrone}$ 2-5: 2×10^{-5} mol L^{-1}; C_{DNA} 1-5: 3.024×10^{-7}, 0, 1.512×10^{-6}, 3.024×10^{-6}, 6.048×10^{-6} mol L^{-1}

Resonance light scattering spectrum of rhein, chrysophanol and physcion interacting with DNA: Lerman demonstrated that acridine dye could insert or intercalate between DNA base pairs. He first proposed the model of DNA intercalator, which is a large class of planar and aromatic molecules and has been found to have the ability to intercalate into DNA in the space between base pairs[17]. A part of anthraquinone material such as adriamycin and mitoxantrone have become the main chemotherapy drugs of cancer and antiviral medicine. Anthraquinone compounds have rigid planar construction. The anthraquinone compounds are exactly twice the length of adjacent bases of single stranded DNA. Therefore, it is easy for them to integrate into the DNA of base pairing or polynucleotide chain of adjacent structures of DNA double helix structure. Physcion, chrysophanol or rhein interaction with DNA results in strong enhanced resonance light scattering signals and it is similar to ethidium bromide adriamycin or mitoxantrone interaction with DNA. Rhein-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 298, 470 and 560 nm (Fig. 4). Chrysophanol-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 360, 470 and 560 nm (Fig. 5). Physcion-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 290, 470 and 560 nm (Fig. 6). We selected the resonance scattering peak at 468 nm for investigation. On the mechanism of ethidium bromide, adriamycin and mitoxantrone interacting with DNA at present, they can parallel intercalate into the base pairs of double-helical DNA. Considering the strong enhanced resonance light scattering signals of physcion, chrysophanol or rhein interacting with DNA is similar to that of ethidium bromide, adriamycin and mitoxantrone, it is presumed that physcion, chrysophanol or rhein is possible to intercalate into the base pairs of double-helical DNA.

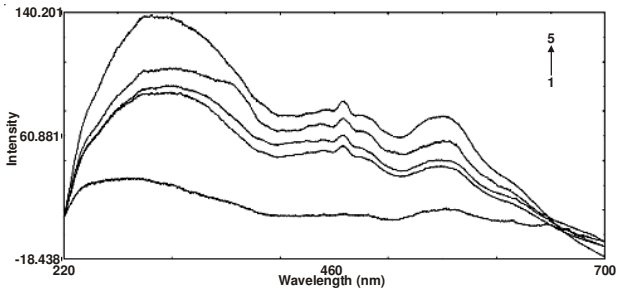

Fig. 4. Resonance light scatting spectra of DNA and rhein. C_{rhein} 2-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-5: 2.268×10^{-7}, 0, 1.512×10^{-7}, 2.268×10^{-7}, 3.024×10^{-7} mol L^{-1}

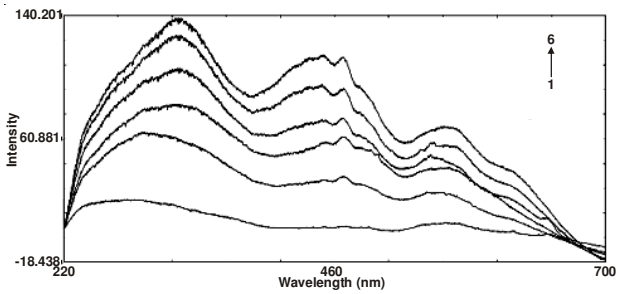

Fig. 5. Resonance light scatting spectra of DNA and chrysophanol. $C_{chrysophanol}$ 2-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-5: 3.402×10^{-7}, 0, 1.512×10^{-7}, 2.268×10^{-7}, 3.402×10^{-7}, 3.78×10^{-7} mol L^{-1}

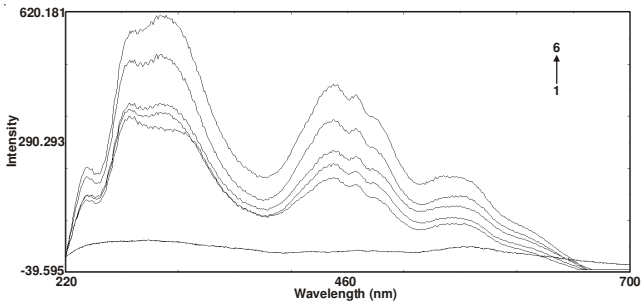

Fig. 6. Resonance light scatting spectra of DNA and physcion. $C_{Physcion}$ 2-6: 1×10^{-6} mol/L 1; C_{DNA} from 1-6: 1.134×10^{-7}, 0, 7.56×10^{-7}, 1.134×10^{-7}, 3.78×10^{-6}, 6.804×10^{-6} mol/L

Saturation value binding with DNA of physcion, chrysophanol, rhein, mitoxantrone, adriamycin interacting with DNA compare with saturation value of ethidium bromide binding with DNA: The number and position of binding site of drug with DNA molecules are the relative determination. When drug concentration is in excess, the resonance scattering signal gradually enhanced with the increasing of concentration of DNA. However, when the DNA reach limit concentration, even if we increase the concentration of DNA, resonance scattering signal will no longer increase. This limit concentration of DNA is on the saturation state. The ratio of drug concentration to saturation concentration of DNA molecular is the saturation value of combination. Therefore, it is given by the following equation. The saturation value of combination = drug molecular concentration/the saturated concentration of DNA. The saturation value of combination can correctly express the ability that drug molecular intercalate the DNA molecular. Therefore, It can serve as a basis of judgement of the strength of the drug. We can calculate saturation value of drug molecular interacting with DNA by the resonance scattering spectra (Table-1). And the saturation value binding with DNA is used for evaluating its potential toxicity of drug. The higher saturation value indicates that drug has greater toxicity. DNA replication, transcription and genetic message expression are delayed or inhibited. We evaluate potential toxicity of rhubarb anthraquinone against ethidium bromide, adriamycin and mitoxantrone through saturation value binding with DNA. Present research find that toxicity of rhubarb anthraquinone is weaker than mitoxantrone, adriamycin and ethidium bromide.

TABLE-1
SATURATION VALUE OF DRUG BINDING WITH DNA

Drug	Saturation value of drug binding with DNA	Saturation value of drug/saturation value of ethidium bromide
Ethidium bromide	14.70	1
Adriamycin	10.58	72.0 %
Mitoxantrone	3.31	22.5 %
Rhein	0.66	4.4 %
Chrysophanol	0.53	3.6 %
Physcion	0.15	1.0 %

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