Multi-spectral analysis of interaction between Shenmai injection and human serum albumin

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Abstract: Shenmai injection is compound traditional medicine and widely employed in adjuvant therapy of cancer patients for improving the patient′s life. The interaction of Shenmai injection with human serum albumin(HSA) in physiological buffer(PH 7.4) was investigated by fluorescence spectroscopy and UV−Vis absorption spectroscopy. These results have significant importance for understanding the pharmacological action pesticide effect of Shenmai injection. Shenmai injection can effectively quench the intrinsic fluorescence of HSA and the results shown that the quenching mechanism was a dynamic process, which was further proved by the UV −Vis absorption spectroscopy. The binding constant K_A at different temperatures (296, 303, 310 K) were obtained from Modified Stern-Volmer analysis of the fluorescence quenching data. The thermodynamic parameters were calculated by Van′t Hoff equation ($\Delta G<0$, $\Delta H>0$, $\Delta S>0$). It indicated that the hydrophobic interactions play an important role in the interaction of Shenmai injection and HSA. In addition, the binding process was spontaneous. Furthermore, the synchronous fluorescence spectra showed that the maximum fluorescence peak of tyrosine residues changed which meant the binding of Shenmai injection to HSA mainly acting on tyrosine residues and the interaction can induce the microenvironment and conformation changes of HSA.

Key words: Shenmai injection; human serum albumin (HSA); fluorescence spectroscopy; UV absorption spectroscopy; quenching mechanism; thermodynamic parameter

CLC number: O433.4 **Document code:** A **DOI:** 10.3788/IRLA201746.1123001

参麦注射液与人血清白蛋白相互作用的多光谱研究

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摘 要: 参麦注射液是一种中药复合物,广泛应用于癌症患者的辅助治疗中。在生理(PH7.4)环境下,通过荧光光谱与紫外吸收光谱研究了参麦注射液与人血清白蛋白的相互作用。 荧光光谱和紫外吸收光谱实验结果表明,参麦注射液能够有效地引起人血清白蛋白的内荧光源淬灭,其淬灭机理为动态淬灭。利用 Stern−Volmer 方程对荧光光谱数据进行分析,得到不同温度(296,303,310 K)下的结合常

收稿日期:2017−03−05; **修订日期**:2017−04−03

基金项目:国家自然科学基金(61377001)

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数(K_A)。通过 Van′t Hoff 方程计算出热力学参数($\Delta G<0, \Delta H>0, \Delta S>0$),该结果表明疏水力是人血清白蛋白与参麦注射液结合时的主要相互作用力及结合过程是一个自发过程。此外,同步荧光光谱实验结果表明,当参麦注射液与人血清白蛋白结合时,主要结合点位于酪氨酸残基,且引起了人血清白蛋白的结构变化。

关键词:参麦注射液; 人血清白蛋白; 荧光光谱; 紫外吸收光谱; 淬灭机制; 热力学参数

0 Introduction

The treatment of tumor with Traditional Chinese Medicine (TCM) is an effective method for human body[1]. Using TCM to treat malignancies have great advantages, such as relieving pain, reducing injury and adverse reactions of the normal cell, which caused by surgery, radiotherapy and chemotherapy and so on. Shenmai injection is a kind of traditional Chinese medicine compound injection. It is composed primarily of red ginseng and radix ophiopogonis[2]. It′s widely used in clinical treatment of tumor patients. When combined with chemotherapeutic agents, it has certain synergies, at the same time the side effects can be reduce caused by chemotherapy drugs treatments, and improves patients´ health, protects the function of marrow hemopoietic. As extensively applied on clinic, to understand the operation mechanism in the body of Shenmai injection can provide reference for its application.

Human serum albumin (HSA) is the most abundant protein in blood plasma. It plays an important role in equilibrium osmotic pressure and transports various molecules in blood[3]. At the same time, it also has good target ability. After the drugs enter human body, drugs will bind with HSA firstly and arrive at receptor site through transportation in blood[4]. Concentration of free drugs, bio distribution and the metabolic process are influenced by the interaction between drugs and HSA significantly. For these reasons, HSA is commonly used as a model protein for biological studies.

Because of its diversified information and high sensitivity, fluorescence spectra were widely applied in the study of interaction between drugs and protein[5]. Fluorescence spectrum can obtain the fluorescence emission peak characteristics, the fluorescence intensity, quantum yield and other information. It′s helpful to analyze the changes of microenvironment around fluorescent chromophores. Therefore, we can get a lot of valuable information about the interaction between protein and drugs through fluorescence spectroscopy.

The interaction of Shenmai injection and HSA was investigated by UV-Vis absorption spectroscopy and fluorescence spectroscopy in simulated physiological conditions. Moreover, the effect of temperature and concentration of drugs were studied by the way of controlling the variable.

1 Materials and methods

1.1 Materials

HSA was purchased from Sigma Chemical Corporation. A 1×10^{-4} mol/L stock solution was prepared by dissolving the solid HSA in 0.5% NaCl, 0.05 mol/L Tris-HCl buffer of PH7.4 and stored at 2-4 ℃. Shenmai injection was obtained from Sichuan Sanjingsanhe pharmaceutical Co.Ltd. As contain a variety of compounds and different from a single chemical constituent, Shenmai injection cannot be in accordance with the molar ratio to discuss. So it adopts according to certain volume ratios configuration. The stock solution was prepared by diluting 6 ml Shenmai injection with Tris-HCl buffer (0.05 mol/L, PH7.4) to 100 ml and stored at 2-4 ℃. Double distilled water was used throughout.

1.2 Apparatus and methods

The fluorescence spectra and synchronous fluorescence spectroscopy were recorded by a Cary

Eclipse spectrofluorometer(Varian, USA) equipped with a 150 W xenon lamp and a thermostat bath. The absorption spectra were obtained on a Cary 60 UV–visible spctrophotometer(Varian, USA). The micropipettes (5–50 μl, 100–1 000 μl) were used for pipetting reagents. A quartz cell of 10mm was used for the measurement.

1.3 Procedures of fluorescence measurement

An exact 2.8 ml portion of 1×10^{-6} mol/L HSA solution was add to a 10 mm quartz cell and then successive titrated 5 μl solution of Shenmai injection, this method won't influence the concentration of HSA and the PH of the whole system. The response time is 20 minutes, the fluorescence of Shenmai injection–HSA system tends to be stable. The fluorescence emission spectra were recorded at different temperatures (296, 303, 310 K) in the wavelength range of 300 to 500 nm and excitation wavelength at 280 nm. The excitation and emission slit widths were set at 5.0 nm. The UV–Vis absorbance spectra of HSA in the present of Shenmai injection were recorded at 303 K.

2 Results and discussion

2.1 Fluorescence characteristics of HSA with Shenmai injection

The Shenmai injection has a weak intrinsic fluorescence[6]. The effect of Shenmai injection on HSA fluorescence intensity is show in Fig.1. It showed obviously that the fluorescence intensity decreased gradually with the increase of Shenmai injection concentration and there was a slight blue shift (from 347 nm to 343 nm) at fluorescence maximum wavelength. This illustrated that the intrinsic fluorescence of HSA was quenched by Shenmai injection.

2.2 Fluorescence quenching mechanism

The quenching mechanism of drugs and HSA usually is classified as dynamic quenching and static quenching[7]. Static quenching is a process that quencher and fluorescence materials generate no fluorescing complex, result in the fluorescence intensity decreasing. Dynamic quenching is caused by quencher and fluorescence materials molecules collide at excited state. Quenching mechanisms can be distinguished by quenching constant in different temperature [8]. For dynamic quenching, the higher temperature lead to larger diffusion coefficients and the quenching constant increased. On the contrary, temperature increasing result in stability of complexes decreased and quenching constants lower in static quenching. To determine the mechanism, the fluorescence quenching data were analyzed by Stern-Volmer equation[9].

$$F_0/F=1+K_q\tau_0[Q]=1+K_{SV}[Q] \quad (1)$$

where F_0 and F are steady-state of fluorescence intensities before and after adding Shenmai injection, respectively. K_q is the quenching rate constant of bimolecular, K_{SV} is the Stern-Volmer quenching constant, $[Q]$ is the concentration of quencher and its value is 10^{-8} s. The Stern-Volmer quenching constant K_{SV} of reaction between HSA and Shenmai injection at different temperature is shown in Fig.2. Table 1 showed the values of K_{SV} and relative constant R at

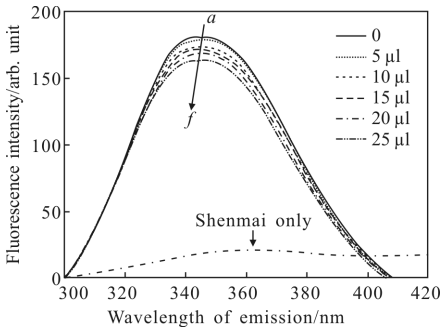

Fig.1 Effect of Shenmai on fluorescence spectra of HSA T=296 K, $C_{Shenmai}$/μl(a–f): 0, 5, 10, 15, 20, 25

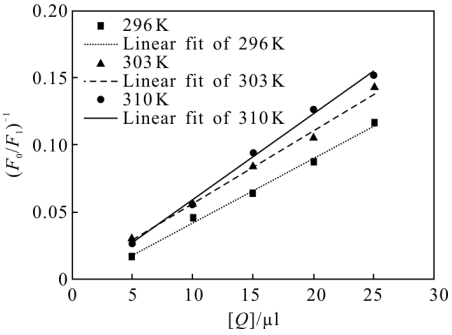

Fig.2 Stern-Volmer curves of Shenmai with HSA at different temperatures

different temperature. K_{SV} increasing with rising temperature indicated that the quenching mechanism of system is dynamic quenching.

Tab.1 Stern-Volmer quenching constants of Shenmai-HSA system at different temperature

T/K	K_{SV}	R
296	0.004 78	0.991 8
303	0.005 42	0.990 7
310	0.006 39	0.995 0

2.3 UV-Vis absorption spectroscopy

In order to further proof the quenching mechanism we analyzed the UV -Vis absorption spectra (Fig.3) Sharma and others consider that dynamic quenching only affects exited states of molecules without changing the absorption spectra of fluorescence materials[10]. From Fig.3, with addition of Shenmai injection, there were no significant changes in absorption spectra. It testified the quenching mechanism of binding of Shenmai injection and HSA is dynamic quenching.

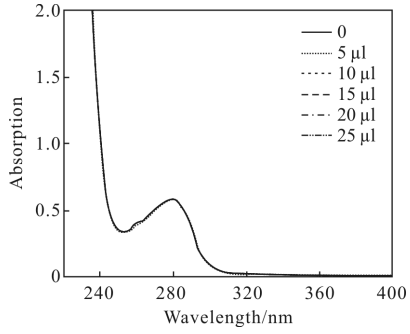

Fig.3 UV-Vis absorption spectra of HSA (T=303 K) in different volumes of Shenmai injection

2.4 Binding constants and interaction force between HSA and Shenmai injection

In the fluorescence experiments, experiment results will be disturbed by fluorescence complex and other kinds of light. So using Modified Stern-Volmer equation can eliminate the influences from these factors[11].

$$\frac{F_0}{\Delta F}=\frac{F_0}{F_0-F}=\frac{1}{f_a K_A}\frac{1}{[Q]}+\frac{1}{f_a} \tag{2}$$

where ΔF is variation of fluorescence intensity, f_a is fraction of accessible fluorescence. K_A is binding constant. Figure 4 shows the linearity between $F_0/\Delta F$ and $1/[Q]$ in Shenmai injection-HSA system. It also can illustrate that rising temperature made the combination of system more stable.

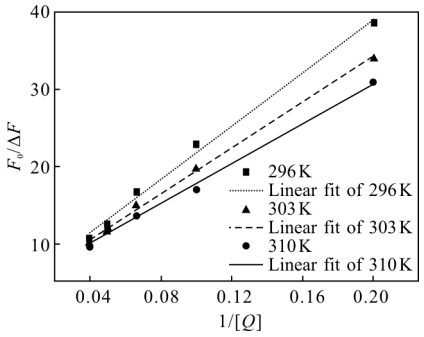

Fig.4 Modified Stern-Volmer curves of Shenmai with HSA at different temperature

Mainly interaction forces between drugs with a bimolecular are hydrophobic force, Vander Waals, electrostatic and hydrogen bonds. Based on plenty of experiment data and analyzing results, Ross and Subramanian concluded that the interaction forces were associate with magnitude of thermodynamic parameters including enthalpy(ΔH) and entropy(ΔS)[12]. The thermodynamic parameters can be determined by using van't Hoff equation.

$$\ln K_A=-\frac{\Delta H}{RT}+\frac{\Delta S}{R} \tag{3}$$

$$\Delta G=-RT\ln K_A=\Delta H-T\Delta S \tag{4}$$

where K_A is binding constant at corresponding temperature T, R is the gas constant and ΔG is free energy. The values of ΔH and ΔS were calculated from the slop and intercept of the van't Hoff plot shown in Tab.2. The values of ΔH and ΔS indicated that

Tab.2 Relative binding constants and thermodynamic parameters of Shenmai-HSA at different temperature

T/K	K_A	ΔH	ΔS	ΔG
296	0.024			$-15.42\times10^3<0$
303	0.030	$23.60\times10^3>0$	131.83>0	$-16.34\times10^3<0$
310	0.037			$-17.27\times10^3<0$

hydrophobic forces have made major contribution to the interaction of Shenmai injection and HSA. Negative ΔG means that this interaction process was spontaneous.

2.5 Effect of drug on HSA conformation

The synchronous fluorescence spectra can provide information microenvironment surrounding the chromophore and it is a useful method to evaluate structure changes of HSA in various concentration of Shenmai injection[13]. When $\Delta\lambda$ between exaction and emission wavelength was set at 15 nm and 60 nm, the synchronous fluorescence spectra provided the microenvironment changes of tyrosine residues and tryptophan residues, respecttively. Synchronous fluorescence spectra of HSA with vary concentration of Shenmai injection were scanned at $\Delta\lambda$=15 nm or 60 nm(Fig.5(a) and (b), respectively). From Fig.5, the

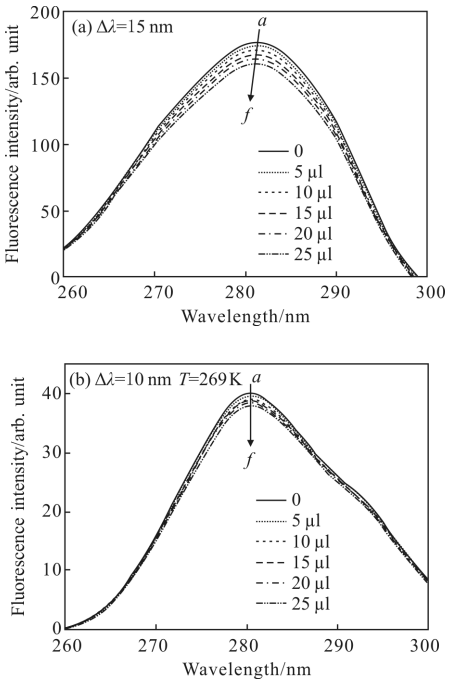

Fig.5 Synchronous fluorescence spectra of HSA with Shenmai injection in different volumes

maximum emission wavelength of tyrosine residues have no obvious shift because the polarity around tyrosine residues not influenced by Shenmai injection. In contrast, the maximum emission wavelength of tryptophan residues has slight blue shift. This phenomenon illustrated that polarity around tryptophan residues decreased and the hydrophobicity increased in presence of Shenmai injection. In this way, drugs can enter hydrophobic pocket and bind tightly with HSA.

3 Conclusion

The interaction between Shenmai injection and HSA was investigated by spectroscopic including fluorescence spectroscopy and UV −Vis absorption spectroscope. The results showed Shenmai injection was a strong quencher. In the interaction between Shenmai injection and HSA, the quenching mechanism was dynamic quenching, the hydrophobic forces were major binding forces and the interaction is spontaneous. In addition, from synchronous fluorescence spectra, the results showed that the structure of HSA was changed after adding Shenmai injection. This study is expected to provide useful information to understand how Chinese traditional medicines work with HAS and clinical application.

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