Interaction Study between Active Components from Traditional Herbal Medicine and Bovine Serum Albumin

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Abstract

Traditional herbal medicine, as a unique form of natural medicine, has been used in traditional therapeutic systems over many years. Active components in herbal medicine are the material basis for the prevention and treatment of diseases. Research on drug-protein binding is one of the important contents in the study of early stage clinical pharmacokinetics of drugs. Plasma protein binding study has far-reaching influence on the pharmacokinetics and pharmacodynamics of drugs and helps to understand the basic rule of drug effects. It is important to study the binding characteristics of the active components in herbal medicine with proteins for the medical science and modernization of herbal medicine. In the present research work some simple and easily available analytical methods have been used to study the active herbal components-protein binding. Rules and influence factors of the binding between different types of active herbal components and proteins are studied.

Keywords: Traditional herbal medicine, Active components, Protein binding, Analytical methods

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I INTRODUCTION

Traditional herbal medicine is the summary of practical experience of people for thousands of years in the fight against disease. It is the treasure of culture and constitutes multi-billion-dollar markets—more than 1500 kinds of herbal medicines are sold as dietary supplements or the raw material of medicines [1]. Its active components are the substantial basis for the treatment of various diseases and the related study is also one of the most important parts of the modernization of herbal medicine.

The concentration of the free active (or toxic) components is directly related to the biological effect (or poisoning), and the concentration of the free drugs in plasma is directly related to the concentration in the tissue. When drugs are absorbed into the blood, drug-plasma protein binding is a common and reversible dynamic process [2]. It is one of the important parameters of drug efficacy and safety, and the determination of bound fraction is a necessary step in drug discovery and clinical trials [3]. It determines the pharmacokinetic and pharmacodynamic characteristics of drugs and influences drug absorption, distribution, metabolism, excretion and toxicity (ADMET) [4,5]. It is generally considered that only free drug can transfer through biological membranes, combine with the appropriate site of action and drive the therapeutic outcome [6]. And then it displays the pharmacological and/or toxicological effects [7]. Small molecular substances can be protected from

some elimination pathways, such as enzymatic reactions in the liver or blood and glomerular filtration of the kidneys, by forming non-covalent complexes with plasma proteins [8]. As a drug reservoir, the bound drug fraction can maintain an effective concentration and prolong the duration of the drug action. For the drugs with high affinity for plasma proteins, they generally need a higher dose to reach therapeutic level, have a long half-life and probably increase toxicity. Conversely, the drugs with low plasma protein binding affinities are limited in their ability to perfuse tissues and reach the site of action [9].

Although many herbal medicines have been proved to be effective by modern clinical trials and pharmacological studies, their active components and the remedial mechanism are still unclear [10]. The pharmacological activities of herbal medicines are considered to be the combination of multi-components effects, including the interactions of active components with proteins. It is well known that a kind of herbal medicine usually contains hundreds of different components [11]. There is no doubt that this is a complex and heavy work to elucidate the mechanism of action of these components. Therefore, it is extremely valuable to investigate the binding of one or a few active components from herbal medicine with proteins.

II EXPERIMENTAL

Materials and apparatus:

A Soxhlet extractor was used for extraction of herbal medicinal plants. Fluorescence measurements were recorded using F-2700 PC Hitachi spectrofluorometer Model F- 2700 (Hitachi, Tokyo, Japan) equipped with a 150W Xenon lamp and a slit width of 5 nm. A 1.00 cm quartz cell and thermostatic cuvette holder was used for measurements. The absorption spectra were recorded on a double beam CARY 50-BIO UV-vis spectrophotometer (Varian, Australia) equipped with a 150 W Xenon lamp and a slit width of 5 nm. A 1.00 cm quartz cell was used for measurements. The pH of solution was measured with Scott Gerate pH meter (CG 804). The viscosity measurements were made with Ostwald's viscometer, immersed in a thermostat water-bath at room temperature. All chemicals used were of analytical reagent grade and were purchased from s.d Fine-Chem, Ltd., India and water (Rankem Ltd., India)

III Procedure

Soxhlet method of ethanol extraction

Plant material can be fresh (for example, a plant leaf) or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area. The plant material should be sufficient to fill the porous cellulose thimble (25 g of thyme in a 25- x 80-mm thimble).

The solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 4 hours. Once the process has finished, the ethanol should be evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask.

Fluorescence quenching study

Based on preliminary investigations, the concentration of plant extract was kept constant at while that of bovine serum albumin (BSA) was varied from 5 to 35 µM. Fluorescence spectra were recorded at three temperatures in the range of 400 - 650 nm upon excitation at 365 nm.

UV measurements

The UV–vis spectra were obtained by scanning the solution on the spectrophotometer in the wavelength range of 300 - 550 nm. Herbal extract concentration was fixed at 150 µM while that of BSA was varied from 5 to 30 µM in the presence of phosphate buffer.

Viscosity measurements

The viscosity measurements of protein (1.0×10^{-3} mol L^{-1}) in the presence and absence of herbal extracts were made in a thermostatic water-bath at 25ºC. The data were presented as $(\eta/\eta_0)^{1/3}$ versus r [12], where η and η_0 are the viscosities of BSA in the presence and absence of herbal extract respectively. Viscosity values were calculated from the observed flow time of BSA containing solutions (t) and corrected for buffer solution (t_0), $\eta = (t - t_0)/t_0$.

IV Results and discussion

Fluorescence studies of herbal extract and BSA

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample [13]. BSA shows strong fluorescence emission peak. On increasing concentrations of herbal extract, the fluorescence emission spectra of BSA decreases.

We have measured the competitive absorbance of protein and herbal extract at the excitation wavelength and observed that both of them do not contribute to the inner filter effect as evident from their very low absorbance values. Hence, there is no inner filter effect. The possible mechanisms of quenching include dynamic quenching, static quenching or both [13,14], dynamic and static quenching could be distinguished based on their differing dependence on temperature. For this, we carried out the quenching studies at different temperatures. For fluorescence quenching, the decrease in intensity is usually described by Stern-Volmer equation [12] as shown below:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = K_q \tau_0 [Q] \text{ ------ (1)}$$

Where F_0 and F denote the steady-state fluorescence intensities in the absence and presence of quencher (BSA) respectively, K_{SV} is the Stern-Volmer quenching constant and [Q] is the concentration of the quencher. Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot F_0 / F against [Q].

Binding studies using UV- visible absorption spectra

UV-vis absorption measurement is a very simple method and applicable to explore the structural change and to know the complex formation. The UV-vis absorption spectra of herbal extract, BSA and the herbal extract-BSA system were also investigated to confirm the probable quenching mechanism. The UV-vis

absorbance intensity decreased regularly with the increasing concentration of BSA indicating that BSA molecules were associated with herbal extract and formed a herbal extract-BSA complex. This confirmed again a static quenching mechanism.

Viscosity Measurements

Viscosity measurement is an effective tool to study the binding mode of small molecules to BSA. A classical intercalation binding demands the space adjacent base pairs to be large enough to accommodate the bound ligand and elongate the double helix, resulting in an increase of BSA viscosity. A non-classical intercalation or a groove mode would reduce the BSA viscosity [15]. The viscosity measurements were taken by varying the concentration ratio of BSA and herbal extract. The relative specific viscosities of BSA depends on the concentration of herbal extract, which increases with the value of [herbal extract]/[BSA]. This indicates a non-classical intercalation mode of binding possibly a groove binding *via* hydrophobic interaction between herbal extract and BSA.

V CONCLUSIONS

The safety and efficacy of herbal medicines have been proven through experience passed on from generation to generation. Herbal medicines experienced the change from the single herb to the compound medicines and had established itself as a relatively independent disciplinary system. The single active component in herbal medicines has developed into a multitude of new drugs. Therefore, studies on the binding of active components in herbal medicines and plasma proteins are of great significance to the guidance and evaluation of new drug development. This article explains some analytical techniques including extraction methods, spectroscopic methods and viscosity method for the study of binding between different types of active herbal components and plasma proteins.

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REFERENCES

[1] Wang ZG, Ren J. Current status and future direction of Chinese herbal medicine. Trends Pharmacol Sci.2002;23:347–348.

[2] Vuiginer K, Veuthey J, Carrupt P, Schappler J. Characterization of drug–protein interactions by capillary electrophoresis hyphenated to mass spectrometry. Electrophoresis. 2012;33:3306–3315.

[3] Musteata FM, Pawliszyn J, Qian MG, Wu JT, Miwa GT. Determination of drug plasma protein binding by solid phase microextraction. J Pharm Sci. 2006;95:1712–1722.

[4] Yang XN, Liu JY, Tang X. Pharmacokinetics of salvianolic acids after intravenous injection, with and without Panax quinquefolium protopanaxadiol saponins, in rats. J Ethnopharmacol. 2008;3:408–414.

[5] Gonciarz A, Kus K, Szafarz M, Walczak M, Zakrzewska A, Szymura-Oleksiak J. Capillary electrophoresis/frontal analysis versus equilibrium dialysis in dexamethasone sodium phosphate-serum albumin binding studies. Electrophoresis. 2012;33:3323–3330.

[6] Lohman JJHM, Merkus FWHM, Rahn KH. Plasma protein binding of drugs. Pharm Weekblad. 1986;8:302–304.

[7] Buscher B, Laakso S, Mascher H, Pusecker K, Doig M, Dillen L, Wagner-Redeker W, Pfeifer T, Delrat P, Timmerman P. Bioanalysis for plasma protein binding studies in drug discovery and drug development: views and recommendations of the European Bioanalysis Forum. Bioanalysis. 2014;6:673–682.

[8] Howard ML, Hill JJ, Galluppi GR, Mclean MA. Plasma protein binding in drug discovery and development.Comb Chem High Throughput Screen. 2010;13:170–187.

[9] Jia HY, Yang GL, Li ZW, Xin PY, Zhao Y, Chen Y. Micellar liquid chromatography with dodecyl dimethyl betaine as an in vitro method for prediction of protein-drug binding. J Chromatogr A. 2007;1143:88–97.

[10] Wen XD, Qi LW, Chen J, Song Y, Yi L, Yang XW, Li P. Analysis of interaction property of bioactive components in Danggui Buxue Decoction with protein by microdialysis coupled with HPLC-DAD-MS. J Chromatogr B Anal Technol Biomed Life Sci. 2007;852:598–604.

[11] Jiang WY. Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science. Trends Pharmacol Sci. 2005;26:558–563.

[12] J. R. Lakowicz, Principles of fluorescence spectroscopy, Plenum, New York, 2006, pp. 278, 281, 283.

[13] C. M. Cortez, J. Cunha-Bastos, *Toxicol. Lett.* 2004, 147, 53.

[14] D. Subbaih, A. K. Mishra, *J. Pharm. Biomedi. Anal.* 2005, 38, 556.

[15] Hao-Yu Shen, Xiao-Li Shao, Hua Xu, Jia LI, Sheng-Dong. Pan *Int. J. Electrochem. Sci.* 2011, 6, 532.