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Spectroscopy study and co-administration effect on the interaction of mycophenolic acid and human serum albumin

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ABSTRACT

Mycophenolic acid (MPA), an immunosuppressor, is always administered in combination with several drugs in clinical therapy, which may alter the binding of MPA to human serum albumin (HSA) and could influence its pharmacological activities. Thus, this study evaluated the interaction between HSA and MPA, as well as investigated the effect of co-administrated drugs on the MPA–protein binary system using fluorescence spectroscopy. Results revealed that MPA has a strong capability to quench the fluorescence of HSA, and the acting forces for the binding are hydrogen bonds and van der Waals forces. Competition on combined administration showed that balofloxacin significantly affects the MPA–HSA interaction, as reflected by the remarkable decrease in fluorescence intensity. Furthermore, cefminox sodium has competitive capability with MPA to some extent, whereas methyl prednisone and amlodipine besylate have a minor influence on the binary system. However, simvastatin has no appreciable effect on the MPA–HSA interaction. In addition, three-dimensional fluorescence spectra and circular dichroism spectroscopy, which were employed to determine the conformation, showed that the binding of MPA with HSA can induce conformation changes in HSA.

1. Introduction

Human serum albumin (HSA) is a major protein component of blood plasma that serves an important function in regulating colloidal osmotic pressure and transporting numerous endogenous compounds, such as fatty acids, hormones, toxic metabolites (e.g., bilirubin), bile acids, and metals. HSA also selectively binds to various drug molecules, alters their pharmacokinetic properties, and significantly affects their biological activity in pharmacology [1,2]. The degree of binding of a drug to a protein can govern its distribution into tissues, its elimination from the body, and its therapeutic or toxic effects. HSA is a single-chain, non-glycosylated polypeptide that contains 585 amino acids with a molecular weight of 66,500 Da. The tertiary structure of HSA contains three structurally similar alpha-helical domains (labeled as I, II, and III), which can be further divided into sub-domains A and B [3,4].

Mycophenolic acid (MPA, Fig. 1) is a metabolite of mycophenolate mofetil with the systematic name 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1, 3-dihydroiso-benzofuran-5-yl)-4-methyl-hex-4-enoic acid; this metabolite has been widely used as an immunosuppressive agent to limit the rejection of transplanted organs [5]. MPA can selectively and reversibly inhibit inosine 5′-monophosphate dehydrogenase (IMPDH), which is a key enzyme in the de novo synthesis of guanosine nucleotides, to contribute to the regulation of the immune system [6–8]. As a routine fist-line immunosuppressive agent used in clinical therapy, MPA is often administered with two or more drugs for complicated transplant patients to prevent operation complications in organic transplantation [9].

Hossain investigated the synergistic inhibition effect of MPA combined with abacavir, didanosine, and tenofovir on the inhibition of HIV-1 replication [10]. Kuypers, who studied the combination of MPA with tacrolimus and corticosteroids in different dosage levels, highlighted that the dynamics of long-term MPA pharmacokinetics in combination with tacrolimus differ according to the daily mycophenolate mofetil dose [11,12]. The experiment of Van Gelder on co-administration of MPA, tacrolimus, and ciclosporin demonstrated that combined administration of tacrolimus and mycophenolate mofetil did not increase MPA exposure, but cyclosporine decreased its exposure [13]. Nowak validated an ultrafiltration system for the reliable measurement of free MPA and evaluated some factors that modulate the free concentration of MPA [14]. In his study, MPA binding with HSA was an important factor that affected the interaction of MPA with both its receptor

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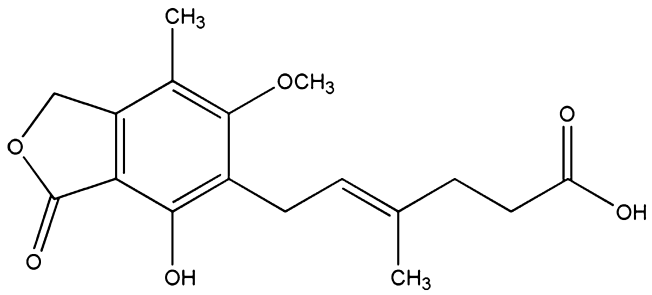

Fig. 1. Molecular structure of MPA.

(IMPDH) and its target cell (PMB). To provide useful information on the competition mechanism of drug combination with protein, Chamai determined the binary and ternary binding of colchicine and lomefloxacin to HSA, given the conclusion that one drug can induce or displace another from its binding site in the HSA molecule [15]. The group of Seedher and Agarwal, who investigated the competitive binding of fluoroquinolone antibiotics and a few other drugs to HSA using fluorescence spectroscopy and circular dichroism, suggested that the presence of a competing drug decreased the binding affinity of fluoroquinolone; they also showed that competitive binding did not cause any major structural changes in the HSA molecule [16]. Investigating drug–protein interactions is an active field to understand the drug action mechanisms and the possibility of designing novel medicine.

In this work, the behavior of MPA–HSA interaction and the effect of the combined administration of balofloxacin, cefminox sodium, amlodipine besylate, methyl prednisone, and simvastatin on MPA–HSA binary system were studied under simulated physiological conditions using fluorescence spectroscopy. The conformational changes in HSA when forming a complex were then investigated by employing circular dichroism and three-dimensional (3D) spectroscopy. In addition, the molecular docking of MPA to HSA was conducted using Discovery Studio 3.1 (DS 3.1, Accelrys Co., Ltd., USA). This study evaluated the competition of combined administration drugs with MPA by fluorescence spectroscopy, clarified the fluorescence quenching mechanism of MPA to HSA for the first time, and investigated the conformational changes in HSA when forming a complex. These are beneficial for the rational use of drugs and may guide the possibility of compound preparation for common co-administrated drugs. It will also bring practical significance in new drug development and pharmaceutical economy.

2. Experimental

2.1. Chemicals

HSA (fatty acid free) purchased from Sigma Aldrich was used directly. The stock solution of HSA was prepared at a concentration of 2.0×10^{-5} mol L^{-1} in 0.1 mol L^{-1} of Tris–HCl buffer solution (pH = 7.40, simulating physiological conditions) containing 0.1 mol L^{-1} of NaCl.

MPA, balofloxacin, amlodipine besylate, cefminox sodium, methyl prednisone, and simvastatin were obtained from Chengdu AiKeda Chemical Technology Co., Ltd. (Chengdu, China). The stock solution of drugs (2.0×10^{-3} mol L^{-1}) was prepared by dissolving them in anhydrous methanol. The final methanol concentration was diluted to 1.0% by volume. The use of 1.0% methanol induced no major HSA structural changes, according to the studies by our group and previous reports [17,18].

All solutions involved in this work were diluted to the required volume with Tris–HCl buffer solution prepared using triple-distilled water, and all the other reagents were of analytical grade. All stock solutions were stored at 0 °C to 4 °C.

2.2. Fluorescence spectroscopic measurements

Fluorescence intensities at three different temperatures (301 K, 309 K, and 316 K) were recorded using a Cary Eclipse fluorophotometer (Varian, USA) equipped with 1.0 cm quartz cells [19,20]. The steady-state fluorescence spectra were recorded in a wavelength range of 300 nm to 500 nm with an excitation wavelength of 280 nm. The slit widths for excitation and emission were set to 5 and 10 nm, respectively. In the measurements, the concentrations of HSA were diluted to 2.0×10^{-6} mol L^{-1}, and ligand concentrations were from 1.0×10^{-6} to 7.0×10^{-6} mol L^{-1} during interaction.

Competition experiments were performed at 301 K by maintaining a ratio of 1:1 for the binary mixture of MPA–HSA. The other drug solutions were then added with increasing molar ratio to the binary system. The fluorescence intensity was determined after maintaining the stability of the ternary system.

Site selective experiments were performed at 301 K using different site probes, namely, WF and ibuprofen for sites I and II, respectively. First, the HSA and probe solution were mixed with similar concentrations of 2.0×10^{-6} mol L^{-1} in the experiment. After the binary system of the HSA–WF/ibuprofen complex was maintained for approximately 15 min, varied concentrations of MPA were gradually added. Fluorescence intensity was recorded under the same experimental conditions as described above.

The 3D fluorescence spectra of HSA (2.0×10^{-6} mol L^{-1}) and the HSA–MPA complexes (1:1 molar ratio) were obtained by using an excitation wavelength ranging from 200 nm to 400 nm with 5 nm increments and monitoring the emission spectra between 200 and 500 nm.

2.3. Circular dichroism spectra

The circular dichroism spectra were recorded on a circular dichroism spectrometer (Model 400, AVIV, USA) in a cell with path length of 2 mm under a nitrogen atmosphere at room temperature. The spectra of HSA and its complex with MPA were recorded at the wavelength ranging from 260 nm to 190 nm with a step size of 1 nm, bandwidth of 1 nm, and average time of 0.5 s. An average of three scans was obtained for all spectra. The HSA concentration was kept constant (1.0×10^{-6} mol L^{-1}), whereas the drug concentrations were 8.0×10^{-6} and 2×10^{-5} mol L^{-1} (ri = [HSA]/[drugs] = 1:8 and 1:20).

2.4. Molecular docking studies

The CDOCKER docking protocol in DS 3.1 was applied to study the interaction between the drug and HSA [21–23]. The structure of MPA was drawn using Material Studio (Accelrys, USA). The structure was optimized. Given the force field, CHARMm was selected. The crystal structure of HSA was obtained from the Brookhaven Protein Data Bank (pdb code: 1h9z). All amino acid residues were repaired, water was removed, and the hydrogen atoms were added to pH 7.4 with DS 3.1 before docking. HSA was defined as a total receptor, and a site sphere was built with a diameter of 10 Å based on WF. The pre-existing WF was then removed. The CDOCKER modular was chosen from the receptor–ligand interaction section of DS 3.1, the operating parameters were set, and the docking process was ran. Ten molecular docking poses were ranked according to −CDOCKER energy ($-E_{CD}$). The pose with the highest $-E_{CD}$ value was chosen as the most suitable mode for the subsequent pose analysis.