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Comparative analysis the binding affinity of mycophenolic sodium and meprednisone with human serum albumin: Insight by NMR relaxation data and docking simulation

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ABSTRACT

Mycophenolic sodium is an immunosuppressive agent that is always combined administration with corticosteroid in clinical practice. Considering the distribution and side-effect of the drug may change when co-administrated drug exist, this paper comparatively analyzed the binding ability of mycophenolic sodium and meprednisone toward human serum albumin by nuclear magnetic resonance relaxation data and docking simulation. The nuclear magnetic resonance approach was based on the analysis of proton selective and non-selective relaxation rate enhancement of the ligand in the absence and presence of macromolecules. The contribution of the bound ligand fraction to the observed relaxation rate in relation to protein concentration allowed the calculation of the affinity index. This approach allowed the comparison of the binding affinity of mycophenolic sodium and meprednisone. Molecular modeling was operated to simulate the binding model of ligand and albumin through Autodock 4.2.5. Competitive binding of mycophenolic sodium and meprednisone was further conducted through fluorescence spectroscopy.

1. Introduction

Mycophenolic sodium (MPS, Fig. 1a), a sodium salt of mycophenolic acid with the trade name myfortic, has been approved for the prevention of acute allograft rejection and widely used as a new immunosuppressive agent to limit the rejection of transplanted organs [1]. Mycophenolic acid can selectively and reversibly inhibit inosine 5′-monophosphate dehydrogenase, a key enzyme in the de novo synthesis of guanosine nucleotides that plays a role in immune system regulation [2–4]. As a routine first-line immunosuppressive agent used in clinical therapy, mycophenolic acid is often administered with two or more drugs for complicated transplant patients to prevent operation complications in organic transplantation. Hossain [5] investigated the synergistic inhibition effect of mycophenolic acid combined with abacavir, didanosine, and tenofovir on inhibition of HIV-1 replication. Van Gelder [6] focused on the co-administration of mycophenolic acid, tacrolimus, and cyclosporin and demonstrated that combined administration of cyclosporine and mycophenolate mofetil may decrease the exposure of mycophenolic acid. Kuypers [7,8] studied the combination of mycophenolic acid with tacrolimus and corticosteroids in different dosage levels and showed the dynamics of long-term mycophenolic acid pharmacokinetics in combination with tacrolimus differs according to the daily mycophenolic acid dose.

Serum albumin acts as a reservoir and transport protein for endogenous compounds, such as fatty acids, hormones, toxic metabolites, bile acids, metals, and exogenous compounds such as nutrients in the blood. Serum albumin also selectively binds to various drug molecules, alters their pharmacokinetic properties, and significantly affects their biological activity in pharmacology [9–12]. The degree of drug binding to a protein can govern its distribution into tissues, elimination from the body, and therapeutic or toxic effects.

Nuclear magnetic resonance (NMR) has been widely used as an effective method to gain insights into the interaction between a ligand and macromolecule because of its ability to measure and analyze abundant information (e.g., chemical shift, relaxation rates, and Nuclear Overhauser Effect [13–18]). NMR relaxation rate technique, which allows for the measurement of selective and non-selective proton spin-lattice relaxation rates, is a powerful tool to

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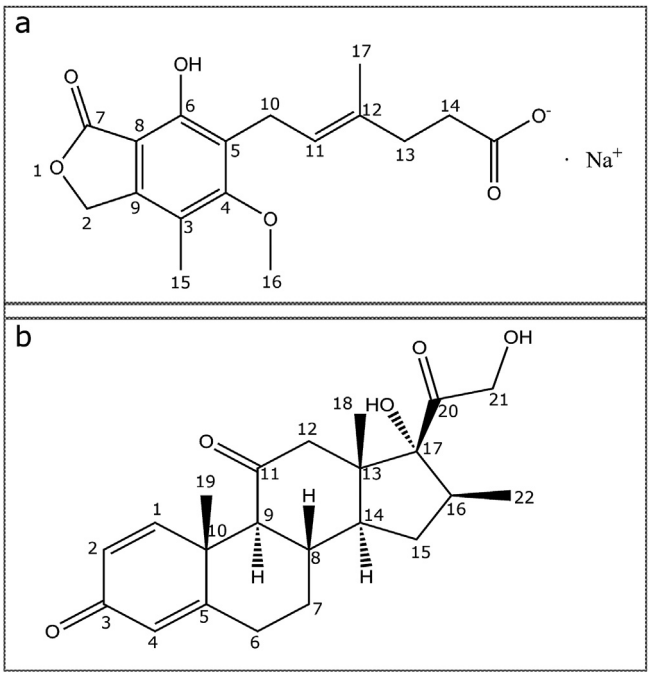

Fig. 1. Molecular structure of (a) MPS and (b) PNE.

investigate the binding affinity of the ligand toward the receptor as well as the dynamic properties of the ligand–protein complex. As a clinical practice, MPS is always combined with corticosteroids to achieve effective and long-term curative effect. The competitive interaction with protein of the combined administrated drugs will affect the distribution and absorption of MPS in the human circulatory system. Our previous studies investigated the influences of co-administrated drugs to mycophenolic acid-human serum albumin (HSA) system by fluorescence spectroscopy methods [19]. In the present study, we further investigated the interaction behavior of MPS and corticosteroid with HSA through NMR relaxation methods. We demonstrated the binding between MPS and HSA and compared their binding ability. Docking simulation was also conducted using AutoDock 4.2.5 to offer a visualized binding model of the ligand-receptor system. Moreover, the interference of corticosteroid to MPS-protein binding system was investigated through fluorescence spectroscopy method. This study aimed to evaluate the binding affinity of MPS and corticosteroid to protein. The results may provide some useful information for understanding the different distribution and side-effect of drug when co-administrated drug exists and may be helpful in the design and modification of the mixed dosage for therapeutic purposes.

2. Materials and methods

2.1. Materials

HSA (fatty acid free) was purchased from Sigma-Aldrich (Milwaukee, USA) and used without further purification. D_2O (99.8% purity) and DMSO (99.9% purity) were provided by Cambridge Isotope Lab. Mycophenolic sodium was prepared and purified in our laboratory. Meprednisone (PNE, Fig. 1b) was purchased from Wuhan Sanhuan Pharmaceutical and Chemical Co., Ltd. (Wuhan, China) and recrystallized in methanol in our laboratory. All other reagents and chemicals were of analytical grade. Ultrapure water was purified using the Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. NMR measurements

^{1}H NMR spectra were obtained with a Bruker Avance 400 MHz spectrometer operating at 400.13 MHz for hydrogen at 298 K. Spin–lattice relaxation rates were measured using the (180°-τ-90°-t)n sequence. The τ values applied for the selective and non-selective experiments were 0.0001, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0, 5.0, and 7.0 s, and the delay time t in this case is 7 s. The 180° selective inversion of the proton spin population was obtained by a selective soft Gaussian perturbation pulse (width: 20 ms, power: 60 dB) corresponding to an excitation width of about 45 Hz. The selective spin–lattice relaxation rates were analyzed using the initial slope approximation and subsequent three-parameter exponential regression analysis of the longitudinal recovery curves.

The solutions for the NMR experiments were obtained by dissolving the appropriate amounts of MPS-protein in D_2O and PNE-protein in DMSO-D_2O (3:1). The solvent mixture was required because of the low solubility of the corticosteroid in D_2O [20]. The protein concentrations used in calculating the affinity index in experiments were 0, 4, 8, 12, and 16 μM; the ligand concentration was constant at 10.0 mM. The affinity index was calculated by linear regression analysis of the experimental data. All NMR data processing and analyses were performed using Topspin 2.5 software (Bruker BioSpin Ltd.).

2.3. Molecular modeling

Molecular modeling of ligand–protein was performed with AutoDock 4.2.5 to identify the potential ligand binding sites. The Lamarckian genetic algorithm implemented in the AutoDock was used to estimate the possible conformations of ligand–protein [21–23]. The available crystal structures of HSA (PDB ID: 1H9Z) were obtained from the Protein Data Bank for docking simulations. In the preparation process, all water molecules and other ligands were removed from the protein PDB file. The Kollman united atom partial charges were assigned, and polar hydrogens were added to the HSA by AutoDock. The three-dimensional form of the ligands were drawn and optimized with Material Studio 6.0 (Accelrys Co. Ltd., USA) and then read in AutoDock software in compatible file format. The ligand roots of MPS and PNE were detected, and the rotatable bonds were then defined. For the recognization of the binding sites in HSA, docking was carried out with setting of grid box size 126 Å × 126 Å × 126 Å along the x, y, and z axes covering whole protein with a grid spacing of 0.697 Å. At first, AutoGrid was run to generate the grid map of various atoms of the ligand and the receptor. After the grid map was completed, ligand flexible docking simulations were performed with 200 runs; 2.5×10^6 energy evaluations; 27,000 numbers of generations; and 150 GA population. After cluster analysis on the docked results, smaller grid maps (47 Å × 41 Å × 53 Å points each with a grid spacing of 0.375 Å centered at coordinates x = 30.938, y = 13.241, and z = 7.96) were defined for further process. A next round of dockings with the number of independent runs set to 100 was performed to determine the optimum binding pose. The conformer with the lowest binding energy of each ligand was used for further analysis.

2.4. Fluorescence spectroscopic measurements

Fluorescence intensities were recorded with a Cary Eclipse fluorophotometer (Varian, USA) equipped with 1.0 cm quartz cells. The wavelength range was chosen from 300 nm to 500 nm, with an excitation wavelength of 280 nm, to record the steady-state fluorescence spectra. The slit widths for excitation and emission were set to 5 and 10 nm, respectively. In the measurements, the HSA