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Characterization of the binding of an anticancer drug, lapatinib to human serum albumin

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ABSTRACT

Interaction of a promising anticancer drug, lapatinib (LAP) with the major transport protein in human blood circulation, human serum albumin (HSA) was investigated using fluorescence and circular dichroism (CD) spectroscopy as well as molecular docking analysis. LAP–HSA complex formation was evident from the involvement of static quenching mechanism, as revealed by the fluorescence quenching data analysis. The binding constant, K_a value in the range of 1.49–1.01 × 10^5 M^{-1}, obtained at three different temperatures was suggestive of the intermediate binding affinity between LAP and HSA. Thermodynamic analysis of the binding data ($\Delta H = -9.75$ kJ mol^{-1} and $\Delta S = +65.21$ J mol^{-1} K^{-1}) suggested involvement of both hydrophobic interactions and hydrogen bonding in LAP–HSA interaction, which were in line with the molecular docking results. LAP binding to HSA led to the secondary and the tertiary structural alterations in the protein as evident from the far-UV and the near-UV CD spectral analysis, respectively. Microenvironmental perturbation around Trp and Tyr residues in HSA upon LAP binding was confirmed from the three-dimensional fluorescence spectral results. LAP binding to HSA improved the thermal stability of the protein. LAP was found to bind preferentially to the site III in subdomain IB on HSA, as probed by the competitive drug displacement results and supported by the molecular docking results. The effect of metal ions on the binding constant between LAP and HSA was also investigated and the results showed a decrease in the binding constant in the presence of these metal ions.

1. Introduction

Cancer is one of the major leading causes of deaths globally. According to the World Health Organization report, the most common cancer incidences were estimated worldwide from those of the lung, breast and large bowel cancers [1]. Lapatinib (LAP), one of the key promising drug molecules, approved by Food and Drug Administration, USA [2] is being used to develop a safe and effective targeted therapy against breast cancer. The clinical efficacy of LAP in combination with capecitabine has been shown effective against HER2-positive breast cancer [3]. Furthermore, LAP-loaded human serum albumin nanoparticles have been proposed to be a safe therapy against HER2-positive cells [4].

LAP (Fig. 1) is a small hydrophobic drug molecule, which belongs to a class of tyrosine kinase inhibitors. This inhibitor is known to suppress the abnormal activity of the human epidermal growth factor receptor 2 (HER2) and the epidermal growth factor receptor (EGFR) by inhibiting their phosphorylation [5–7]. Although, LAP has been shown to be 99% bound to human serum albumin (HSA) and α-1 acid glycoprotein in the blood circulation [8], its binding characteristics in terms of binding mode, binding affinity, forces involved and binding site on these proteins remained unclear.

HSA is the major transporter of many drugs in blood circulation. Three well known drug binding sites I, II and III, located in subdomains IIA, IIIA and IB of HSA, respectively, have been characterized [9–11]. Since pharmacokinetic and pharmacodynamic properties of many drugs depend on their binding affinities with serum proteins in the human body [12], binding characteristics of LAP to HSA need to be explored. Only free drug can diffuse from blood to tissue or organ where the pharmacological activity of drug occurs. In drug–protein interaction, the reversible binding of drug to protein controls the active concentration of drug molecule [13,14]. Such studies are important as low binding affinity of a drug to the protein may result in its poor distribution and short life-span in the blood, whereas high binding affinity interferes with the desired drug efficacy [15]. Hence, investigation on the binding affinity of LAP to HSA may provide useful information about the design of dosage forms [16]. This paper deals with the binding studies of LAP to

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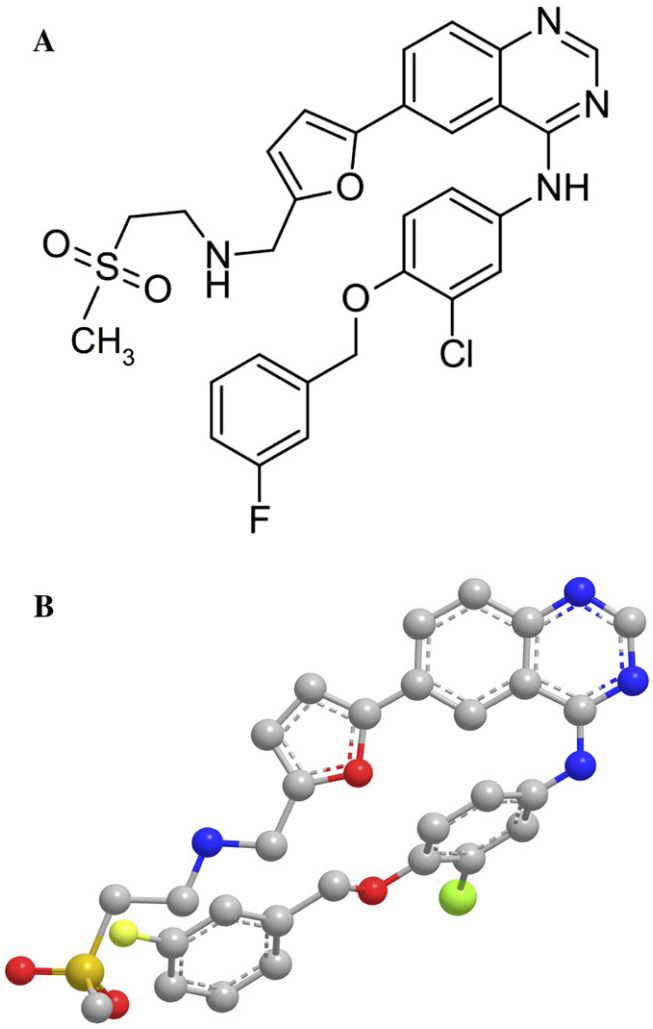

Fig. 1. Structural representation of LAP. (A) Chemical structure and (B) ball-and-stick model.

HSA based on fluorescence and circular dichroism (CD) spectroscopic as well as molecular docking results.

2. Materials and Methods

2.1. Materials

Human serum albumin (HSA), essentially fatty acid free (Lot # 068K7538V), phenylbutazone (PBZ), hemin (HMN) and digitoxin (DGT) were procured from Sigma-Aldrich Inc., St. Louis, MO, USA. Lapatinib (LAP) was the product of Cayman Chemical Company, Michigan, USA. Diazepam (DZM) was purchased from Lipomed AG, Arlesheim, Switzerland. All other chemicals used were of analytical grade purity.

2.2. Analytical Procedures

The stock solution of HSA was prepared by dissolving a known amount of the protein in 60 mM sodium phosphate buffer, pH 7.4 and its concentration was determined spectrophotometrically using a molar extinction coefficient of 36,500 M^{-1} cm^{-1} at 280 nm [17].

Stock solutions of LAP, PBZ, DZM, DGT and HMN were prepared by dissolving known amounts of their crystals in appropriate volume of dimethylsulfoxide (DMSO). The working solutions of the above ligands were made by diluting the stock solutions with 60 mM sodium phosphate buffer, pH 7.4 to the desired volume. The final concentration of DMSO in all experiments was less than 1%.

All experiments were performed in 60 mM sodium phosphate buffer, pH 7.4 and at 25 °C.

2.3. Fluorescence Spectroscopy

The fluorescence spectra of HSA (3 μM) in the absence and the presence of ligand were measured in the wavelength range, 300–400 nm upon excitation at 295 nm on a Jasco FP-6500 spectrofluorometer using a quartz cuvette of 1 cm path length, positioned in a thermostatically-controlled water-jacketed cell holder. The excitation and emission bandwidths were manually set at 10 nm each, while the data pitch and the scan speed were fixed at 1 nm and 500 nm min^{-1}, respectively.

Three-dimensional (3-D) fluorescence spectra of HSA (3 μM) were obtained both in the absence and the presence of LAP (LAP/HSA molar ratio of 1:1) using the excitation and the emission wavelength ranges of 220–350 nm and 220–500 nm, respectively, with 5 nm increments.

2.4. LAP–HSA Interaction Studies

2.4.1. Fluorescence Quenching Titration

The interaction of LAP with HSA was studied by fluorescence quenching titration method as described earlier [18]. In short, a fixed concentration of HSA (3 μM) was titrated with increasing concentrations of LAP (0–4.5 μM with 0.5 μM intervals) in a total volume of 3.0 ml. After an incubation time of 1 h at a fixed temperature, the fluorescence spectra were recorded in the wavelength range, 310–380 nm upon excitation at 295 nm.

In order to investigate the quenching mechanism as well as thermodynamics of the binding reaction, the titration experiments were carried out at three different temperatures, i.e., 288, 303 and 318 K. The obtained fluorescence data were corrected for the inner filter effect, using the following equation [19]:

$$F_{cor} = F_{obs} 10^{\left(\frac{A_{ex}+A_{em}}{2}\right)} \quad (1)$$

where F_{cor} is the corrected fluorescence intensity; F_{obs} is the measured fluorescence intensity; and A_{ex} and A_{em} are the changes in the absorbance values of the protein upon ligand addition at the excitation (295 nm) and the emission wavelengths (300–400 nm), respectively.

2.4.2. Quenching Mechanism

Investigation of the quenching mechanism involved in LAP-induced quenching of HSA fluorescence was made by the temperature dependency of the fluorescence quenching. The fluorescence data were treated according to the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q] \quad (2)$$

where F_0 and F represent the fluorescence intensities of the protein in the absence and the presence of the quencher (LAP), respectively; K_{SV} is the Stern–Volmer quenching constant and [Q] is the quencher concentration [19].

Values of the bimolecular rate constant, k_q of the fluorescence quenching process at different temperatures were obtained by substituting the value of τ_0, the average lifetime of the biomolecule without any quencher as 6.38×10^{-9} s for HSA in the above equation [20].