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Interaction of an antiepileptic drug, lamotrigine with human serum albumin (HSA): Application of spectroscopic techniques and molecular modeling methods

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ABSTRACT

Lamotrigine (an epileptic drug) interaction with human serum albumin (HSA) was investigated by fluorescence, UV–Vis, FTIR, CD spectroscopic techniques, and molecular modeling methods. Binding constant (K_b) of 5.74×10^3 and number of binding site of 0.97 showed that there is a slight interaction between lamotrigine and HSA. Thermodynamic studies was constructed using the flourimetric titrations in three different temperatures and the resulted data used to calculate the parameters using Vant Hoff equation. Decreased Stern Volmer quenching constant by enhanced temperature revealed the static quenching mechanism. Negative standard enthalpy (ΔH) and standard entropy (ΔS) changes indicated that van der Waals interactions and hydrogen bonds were dominant forces which facilitate the binding of Lamotrigine to HSA, the results were confirmed by molecular docking studies which showed no hydrogen binding.

The FRET studies showed that there is a possibility of energy transfer between Trp214 and lamotrigine. Also the binding of lamotrigine to HSA in the studied concentrations was not as much as many other drugs, but the secondary structure of the HSA was significantly changed following the interaction in a way that α-helix percentage was reduced from 67% to 57% after the addition of lamotrigine in the molar ratio of 4:1 to HSA. According to the docking studies, lamotrigine binds to IB site preferably.

1. Introduction

Human Serum albumin (HSA) as the main transporter protein in human circulatory system, plays an important role in the storage and transportation of numerous compounds (e.g. drugs, toxins …) [1]. Binding of drugs to the HSA affects their pharmacokinetic and pharmacodynamic properties. Free portion of drugs can inactively diffuse through the membranes, biliary excretion or glomerular filtration in kidney, distribute via intracellular specific transport systems (e.g. receptor-mediated endocytosis, protein-mediated transport), and interact with therapeutic targets (e.g. receptors) [2]. The tissue unbound concentration (free drug concentration) is in proportion to the plasma unbound concentration of drug which is affected by albumin binding [3].

HSA is a single-chain (Fig.1), non-glycosylated polypeptide that contains 585 amino acids with a molecular weight of 66,500 Da, where the polypeptide chain forms a heart-shaped structure with an approximate dimension of 80 × 80 × 30 Å [4].

Using a fluorescent probe displacement method Sudlow [5] showed the presence of two specific drug binding sites. Site I (located in subdomain IIA) is selective binding site for heterocyclic anions, while site II (located in subdomain IIIA) is selected by aromatic carboxylates. Warfarin, and ibuprofen, are interacted with site I and site II, respectively as stereotypical ligands. Secondary binding clefts have been found for ibuprofen and warfarin to be located on domain II and I, respectively (Fig. 1) [5–7].

In addition to the binding sites I and II, the hydrophobic, D-shaped cavity in subdomain IB as site III is also preferred by some compounds including bilirubin, steroid antibiotic fusidic acid, Schiff base complexes and a sulfonamide derivative [8].

Lamotrigine (Fig.2) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorders. It is used to treat focal seizures, primary and secondary tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome. It is approved for the maintenance treatment of bipolar type I and as an effective mood stabilizer [9]. It has a total protein binding of 55% [10]. No investigation was performed to explore its binding to HSA until now.

Lamotrigine binding to HSA is studied using multi-spectroscopic (UV–Visible, Fluorescence, FTIR, CD) and molecular modeling methods

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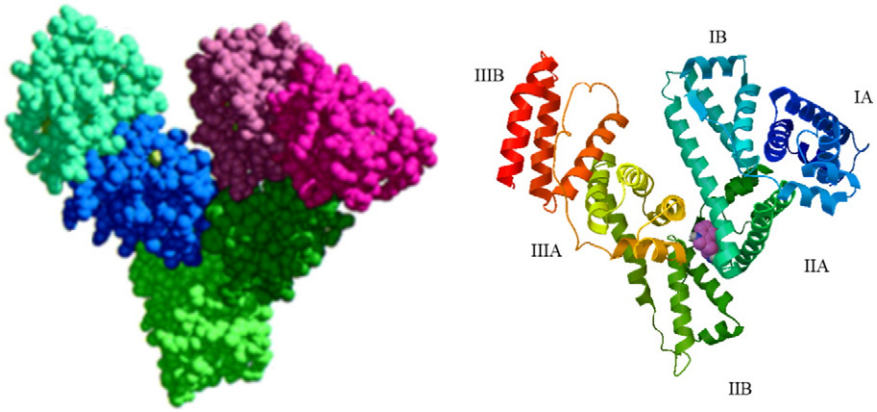

Fig. 1. Crystal structure of HSA and binding sites.

in the present work. The binding parameters (binding constant, number of binding sites, binding distance) along with thermodynamic properties have been evaluated by fluorescence quenching and UV–Vis methods [11–14]. The binding mechanism and HSA conformational alteration were further studied using FTIR and CD methods. Molecular interactions were characterized using molecular docking studies and the results were compared with experimental results.

2. Material and Methods

2.1. Material

Lamotrigine (Arastoo pharmaceutical (Iran)) and Fatty acid free HSA (Sigma) possessed purity >97%. Ethanol (99.9%), NaH_2PO_4 were purchased from Merck. The Stock solutions of Lamotrigine and HSA were prepared in 0.1 M phosphate (NaH_2PO_4) buffer (pH 7.4) containing 0.15 M NaCl. All other materials were of analytical grade.

2.2. Methods

2.2.1. Absorption Spectroscopy

A double beam Shimadzu 1800 (Shimadzu, Tokyo, Japan), were used to study the spectroscopic properties of free drug, HSA and complex in the range of 200–400 nm, at 298 K using a slit of 5 nm and a scan speed of 250 nm min^{-1}. Quartz cuvettes of 1 cm were used for measurements.

2.2.2. FTIR-ATR Spectroscopic Measurements

FT-IR spectra of free HSA and Lamotrigine-HSA complex in the range of 400–4000 cm^{-1} at 298 K were recorded using a Nicolet-6700 FTIR spectrometer equipped with the attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. The resolution for all measurements was 4 cm^{-1} and 64 scans. The corresponding absorbance contributions of buffer and free lamotrigine solutions were digitally subtracted.

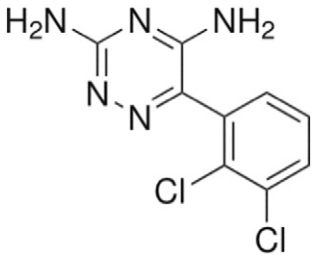

Fig. 2. Molecular structure of Lamotrigine.

2.2.3. Circular Dichroism (CD)

CD Spectra of free HSA (0.125 μM) and complexes (various lamotrigine concentration of 0.125, 0.25 and 0.5 μM) were obtained by a CD Spectrometer (Aviv, USA). A quartz cell with a path length of 0.01 cm was used to do measurements in the far-UV region (190–260 nm) in a nitrogen atmosphere under constant temperature conditions (i.e. 25 °C kept constant using a Neslab RTE-111 circulating water bath connected to the water-jacketed quartz cuvettes). Spectra were corrected for buffer signal and converted to the Mol CD (Δε) using the Jasco Standard Analysis software.

2.2.4. Fluorescence Quenching Spectroscopy

A Jasco spectrofluorometer, equipped with a 150 W Xenon lamp was applied for fluorescence titration data recording. The fluorescence quenching data of HSA (6.66×10^{-6} M) at increasing concentration of lamotrigine (2.0×10^{-5} to 4.0×10^{-4} M) was recorded in the wavelength range 300–450 nm after exciting at 280 nm (slit widths were 10 nm/10 nm). To evaluate the complexation thermodynamic the experiments were repeated at different temperatures; i.e. 288, 298 and 308 K. The instrument was thermostatically controlled by a Neslab RTE-110 circulating water bath.

2.2.5. Molecular Modeling Studies

Crystal structure of HSA (PDB ID:1H9Z) obtained from Protein Data Bank [15]. Three-dimensional (3D) structure of lamotrigine was drawn, and its geometry was optimized using HyperChem 8.0 software.

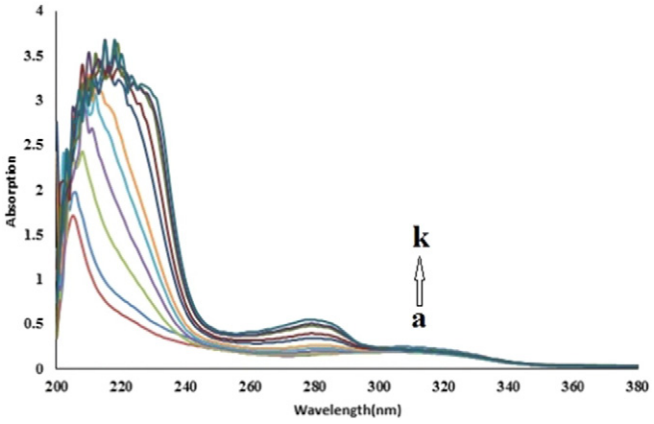

Fig. 3. UV absorption spectra of HSA–lamotrigine system: HSA only 6.66 μM (a); added concentrations of lamotrigine (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM (b–k)) and HSA concentration is constant;