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Study on the interaction of the epilepsy drug, zonisamide with human serum albumin (HSA) by spectroscopic and molecular docking techniques

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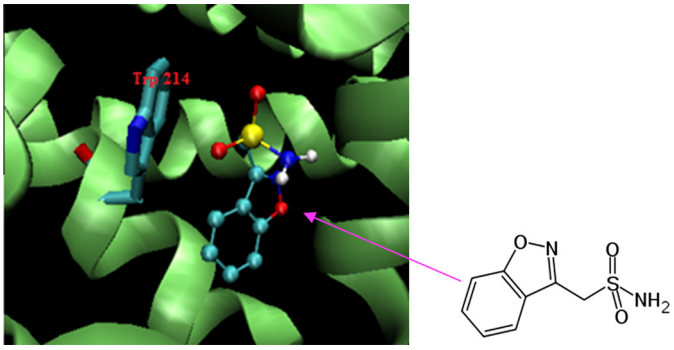
HIGHLIGHTS

- There is evidence of conformation changes of HSA induced by its interaction with zonisamide drug.
- We conclude that, the drug binds to HSA with a high affinity and transport in the body.
- Molecular docking also indicated that zonisamide could strongly bind to the site I (subdomain IIA) of HSA.

GRAPHICAL ABSTRACT

In this study, an attempt has been made to study the interaction of zonisamide with human serum albumin (HSA) employing UV–vis, fluorometric, circular dichroism (CD) and molecular docking techniques.



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ABSTRACT

In the present investigation, an attempt has been made to study the interaction of zonisamide (ZNS) with the transport protein, human serum albumin (HSA) employing UV–Vis, fluorometric, circular dichroism (CD) and molecular docking techniques. The results indicated that binding of ZNS to HSA caused strong fluorescence quenching of HSA through static quenching mechanism, hydrogen bonds and van der Waals contacts are the major forces in the stability of protein ZNS complex and the process of the binding of ZNS with HSA was driven by enthalpy ($\Delta H = -193.442$ kJ mol^{-1}). The results of CD and UV–Vis spectroscopy showed that the binding of this drug to HSA induced conformational changes in HSA. Furthermore, the study of molecular docking also indicated that zonisamide could strongly bind to the site I (subdomain IIA) of HSA mainly by hydrophobic interaction and there were hydrogen bond interactions between this drug and HSA, also known as the warfarin binding site.

Introduction

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) is a sulfonamide antiepileptic approved for use as an adjunctive therapy in adults with partial-onset seizures [1]. Zonisamide is an FDA-approved drug for use in treating epilepsy. It has many actions which include blocking voltage-dependent sodium channels, inactivating T-type calcium channels and reducing calcium influx during membrane depolarization, actions complicated with preventing seizure spread [2–4]. ZNS also weakly inhibits carbonic anhydrase (CA) activity [5]. It has also been used off-label by psychiatrists as a mood additive to treat bipolar depression [6]. It is also reported that zonisamide improves clinical result when used as an adjuvant in therapy for Parkinson's disease (PD) [7] possibly due to its ability to transform dopamine (DA) neurotransmission.

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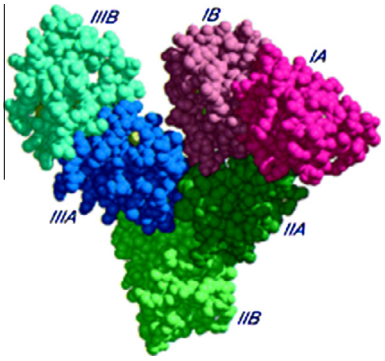

Fig. 1. Crystal structure of HSA.

ZNS is generally safe and well abided at therapeutic doses. The most commonly reported adverse effects associated with ZNS use were somnolence, anorexia, dizziness, headache, nausea, and agitation–irritation in a retrospective case study [8].

Human serum albumin (HSA) (Fig. 1) is the most abundant protein constituent of blood plasma and serves as a protein stowing component. The three-dimensional structure of human serum albumin has been determined through X-ray crystallographic measurements [9]. This globular protein consists of a friendless polypeptide chain of 585 amino acid residues, which has many important physiological functions [10]. HSA considerably contributes to colloid osmotic blood pressure and realize transport and distribution of many molecules and metabolites, such as fatty acids, amino acids, hormones, cations and anions, metal ions, and many diverse drugs. HSA can bind and carry through the bloodstream many drugs, which are poorly soluble in water. It has been exposed that the distribution, free concentration and the metabolism of various drugs can be significantly altered as a result of their binding to HSA [11]. The X-ray crystallographic studies reveal that the heart shaped HSA consists of three architecturally similar domains (I, II and III), each of which contains two subdomains (A and B) [12,13]. These subdomains are predominantly helical and extensively cross-linked through several disulfide bridges, with one tryptophan residue (Trp214) in subdomain IIA [14,15]. It is suggested that the principal regions of ligand binding to HSA are situated in hydrophobic cavities in subdomains IIA and IIIA, which are designated as sites I and II, respectively [16,17]. In this study, we have reported the conformational changes of HSA in the presence of zonisamide (Fig. 2) using circular dichroism (CD) spectroscopy, UV–Vis and fluorescence spectroscopy. The aim of our work is to disclose the mechanisms of interactions of zonisamide with HSA through the thermodynamic thoughts. Also zonisamide molecule has been docked into the 3D structure of HSA in order to envisage a connection between the experimental and theoretical results.

Material and methods

Material

Zonisamide, Human serum albumin (HSA) was purchased from Sigma and Ethanol (99.9%), Na_2HPO_4, NaH_2PO_4, Warfarin and Ibuprofen were purchased from Merck.

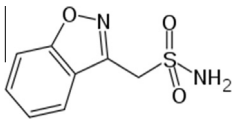

Fig. 2. Molecular structure of zonisasmide.

Preparation of stock solutions

Zonisamide and HSA solutions were prepared in the buffer solution adjusted to pH 7.4 with 0.1 M Na_2HPO_4 and NaH_2PO_4, containing 5% (w/w) ethanol in order to overcome the low solubility of the zonisamide in pure aqueous medium. HSA stock solution (10^{-3} M, based on its molecular weight of 66,000) was prepared in 0.01 M phosphate buffer of pH 7.4 and was kept in the dark at 4 °C. Triple distilled water was used throughout the experiment.

Methods

Fluorescence spectra

All fluorescence spectra were recorded with a LS-55 Spectrofluorimeter (Perkin–Elmer corporate, UK) equipped with a Xenon ampuxl-159, quartz cells (1.0 cm) and a thermostat bath. The maximal fluorescence emission of HSA at λ_{ex} = 290 nm was located at 346 nm. Fluorescence spectra were recorded at 283, 298 and 310 K in the range of (290–470 nm). The spectra band widths of excitation and emission slits were both kept at 5.0 nm. The fluorescent intensities at 346 nm were recorded with the excitation wavelength of (290 nm).

UV-spectrophotometry

Absorbance spectra were recorded in the range (200–500 nm) using an HP spectrophotometer (Agilent 8453), equipped with a thermostated bath (Huberpolysatcc1). Absorption titration experiments were carried out by keeping the concentration of HSA constant (3×10^{-5} M) while varying the drug concentration from 0 to 4.5×10^{-5} M (r_i = [ZNS]/[HSA] = 0.0, 0.16, 0.3, 0.4, 0.5, 0.67, 0.77, 0.86, 0.95, 1, 1.1, 1.2, 1.25, 1.3, 1.35, 1.4, 1.5, 1.53). Aliquots of the HSA solution were treated with the zonisamide at several input molar ratios (r_i). Absorbance values were recorded after each successive addition of drug solution and equilibration (ca.5 min).

Circular dichroism studies

Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter (200–250 nm) and cell length path was (0.1 cm) by keeping the concentration of HSA constant (3×10^{-6} M) while varying the drug concentration from 0 to 4.2×10^{-6} M (r_i = [ZNS]/[HSA] = 0.0, 0.7, 1.2, 1.4).

Molecular docking

The known crystal structure of HSA (PDB Id: 1AO6) was obtained from the Brookhaven Protein Data Bank. The three-dimensional (3D) structure of ZNS was built, and its geometry was optimized through Hyper Chem Professional program.

Auto Dock generated the different ligand conformers using a Lamarkian GA, a GA implementation with an adaptive local method search [18]. The simulations started with a predefined number of generation cycles, composed of mapping and fitness evaluation, selection, crossover, mutation and elitist selection steps, and continued with a local search, followed by the scoring of the generated conformers. The energy-based Auto Dock scoring function includes terms accounting for short range van der Waals and electrostatic interactions, loss of entropy upon ligand binding, hydrogen bonding, and solvation. The protein and the ligand input structures, prepared as described above, were transformed into the corresponding pdbq format files (containing atom coordinates, partial charges, and solvation parameters). To recognize the binding sites in HSA, blind docking was carried out, the grid size set to 60, 60, and 60 along the *X*, *Y*, and *Z* axes with 0.375 Å grid spacing.

The center of the grid set to 22.210, 8.152, and 4.149 Å. The Auto Docking parameters used were GA population size: 250 and maximum number of energy evolutions: 250,000. The lowest energy conformation was used for further analysis.