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Short Communication

INVESTIGATING THE BINDING INTERACTIONS OF NADOLOL WITH HUMAN SERUM ALBUMIN- A COMBINED THERMODYNAMIC AND MOLECULAR MODELLING APPROACH

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ABSTRACT

Nadolol is a beta blocker, commonly used in the cure of arterial hypertension. In order to accomplish an optimal performance and efficacy of this drug molecule, it is important to consider the possible complexation of nadolol with human serum albumin. As the degree of interaction of a drug to plasma proteins has a visible effect on its delivery, elimination and pharmacological effect as only the unbound part of drug is available for distribution into extra-vascular part. In this work, we have chosen Isothermal titration calorimetry and Molecular docking techniques to investigate the binding interactions of Nadolol with human serum albumin. The ITC data revealed that the binding between nadolol and Human Serum Albumin was through entropy driven route and the association constant K_a=8.73×10^{6}M^{-1}and the results obtained by ITC were complemented with *in-silico* studies.

Keywords: Nadolol, Human serum albumin, Isothermal titration calorimetry, Docking

1. INTRODUCTION

Nadolol (Fig. 1) is an anti-hypertensive, non-selective β -adrenergic antagonist and with no partial agonist activity [1]. Nadolol is advised once daily; it is only known beta blocking drug which has longest half-life in plasma [2]. It is non cardio selective, is not metabolized in liver, is helpful in reduction of hypertension, and during cirrhosis, it also protects from gastrointestinal bleeding [3-5].

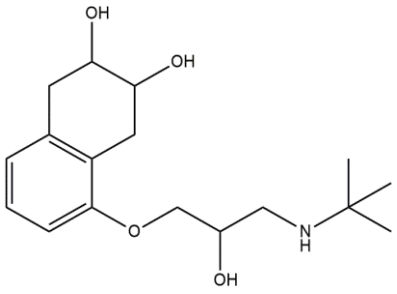

Fig. 1: Chemical structure of Nadolol

Nadolol itself has different characteristics compared to other beta-blockers as it has 17 to 24 hours half- life in adults compared to 4 to 6 hours in case of propranolol and moreover nadolol is excreted unchanged by the kidney. In addition, nadolol is freer or active due to this it is less desirable during pregnancy [6].

Human serum albumin (HSA) is a most abundant transporter protein among plasma proteins which helps in carrying the drugs or other ligands. The physiological metabolism and distribution of drug also depend upon how the drug binds to transporter protein [7]. Hence, interaction or complexation studies of drugs with plasma proteins help to recognize the binding mechanism.

Human serum albumin (1BM0 chain A) [8] (Fig. 2) the main carrier protein is single polypeptide chain accommodating 585 amino acids residues which is divided into three homologous domains; domain I, domain II and domain III; further each homologous domain is subdivided into Sub-domain A and Sub-domain B. Major binding sites for most of drugs or ligands are Subdomain IIA and Subdomain IIIA which are referred as Sudlow site I and Sudlow site II respectively [8-9]. Total seventeen disulphide bonds are there in HSA and at Sudlow's site I single tryptophan residue (Trp-214) present and Tryptophan residues are the main intrinsic fluorophores which are very sensitive to their micro surrounding changes. It is difficult to study plasma protein interaction due to lack of

specificity toward particular drugs, large number of HSA binding sites and allosteric effects. Yet, there are large number of methods to study drug plasma protein binding have been developed [10]. Drug reaction with protein is a reversible reaction by involving various weak interactions like hydrophobic interactions, van der Waals interactions, ionic interactions and H- bonding. Binding of drug on HSA takes place on carboxyl group, -OH group and other available groups on the amino acid residues [11].

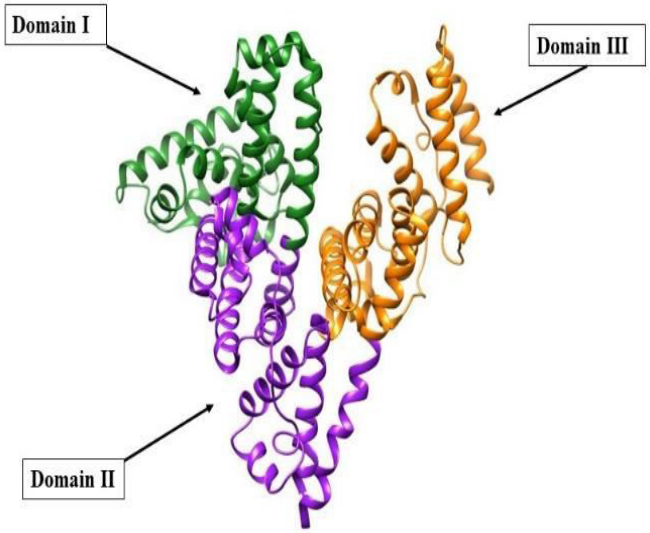

Fig. 2: 3D image of Human serum albumin HSA (PDB id: 1BM0_chain- A)

Plasma protein complexation with drugs can be studied by different methods like separative, non- separative and other methods to understand binding parameters of drug and proteins. Separative methods includes equilibrium dialysis which is very common in pharmaceutical industries but this process is time consuming, difficult to automate, labour intensive and costly as well, moreover small molecule binding to the container may also distress the results obtained. Ultrafiltration method is also been used, which is simple and fast method but again binding of nonspecific molecules to the filtration unit can be big issue with this technique. Other separative techniques are like Liquid chromatography-mass spectrometry method with immobilized HSA column, capillary electrophoresis has been used with disadvantages that while separation of free drug there is a disturbance in drug- protein equilibrium. Non-Separative methods include calorimetric methods such as differential scanning calorimetry and isothermal calorimetry. Furthermore, Circular dichroism and fluorescence give higher throughput [12]. Molecular modelling studies are also conceded to characterize the changes in conformations of HSA due to interaction with drug. The present research is aimed at the investigation of the HSA-Nadolol binding with by isothermal Calorimetric method and further, reinforcement of the experimental study using computational methods like docking. The detailed over view of the drug-protein interaction will be predicted by molecular docking studies and the predicted molecular modelling results will afterwards be confirmed by results of ITC Studies.

2. EXPERIMENTAL

2.1. Material

Free from essential fatty acid Human Serum Albumin and Nadolol were purchased from Sigma Chemical Co. Bangalore, India. The solutions were prepared in sodium phosphate buffer of pH 7.4. The concentration of HSA stock solution was determined through UV-visible spectrophotometer using extinction coefficients (ε) 36600 $M^{-1}cm^{-1}$ at 280 nm. A stock solution of Nadolol (150 μM) was prepared in sodium phosphate buffer of pH 7.4 and further samples of drug of different concentrations were prepared in the stock dilution method. The chemicals used were of analytical grade and sample preparation was done by using doubly distilled and degassed water.

2.2. Methods

2.2.1. Isothermal titration calorimetric studies

In order to determine magnitude of binding affinity (K_a), contributing binding energy terms *i.e.* change in enthalpy(ΔH^o) and change in entropy(ΔS^o) and stoichiometry (N) of Nadolol with HSA; Isothermal Titration calorimeter (ITC200 micro calorimeter, Micro Cal Inc., Northampton, MA) was employed. The temperature and pH conditions were kept at 298K and 7.4, respectively. The solutions were degassed thoroughly before use for 20 min. Reference reaction cell was loaded with sodium phosphate buffer and sample cell was loaded with HSA (1μM) and Nadolol (1 mM) was injected into the reaction cell by rotating syringe. To examine the effect of nadolol, its concentration was increased into the reaction cell comprising HSA. Each titration consisted of 20 consecutive injections of 1 mM nadolol solution into the sample reaction cell containing 10 μM HSA solution. Further, same concentration of drug was used to perform control experiment to correct the data for

heats of dilution of the ligand, protein and buffer mixing. This will give the heat of binding reaction between drug and protein which is a difference of heat of reaction and the corresponding heats of dilutions. The data were analysed by using Origin 7.0 software provided with the instrument.

2.2.2. Molecular docking studies

Molecular modelling was performed through Autodock 4.2 [13] The 3D Structure of HSA (PDB ID: 1BM0) was downloaded from the PDB [8]. The structure of Nadolol (pub chem id: 39147) was developed using the Charmm force field.[14] Water molecules were removed and polar H- atoms were added. Lamarckian genetic algorithm was employed for docking calculations and default values of all other parameters were used. Twenty different docked model conformations with low binding energy values were selected and out of these 20 different conformations, the best docked model with lowest binding energy value was selected and was used for analysis.

3. RESULTS AND DISCUSSION

3.1. Thermodynamic parameters analysis by Isothermal Calorimetric Titration

Isothermal Titration Calorimetry is an efficient and important technique to determine the thermodynamic binding profile of Drug- Protein interaction and it is considered more reliable than spectroscopic techniques to determine thermodynamic parameters [15]. The data obtained were analysed by an independent binding model to determine the thermodynamic information of binding *i.e.* Association constant (K_a), the stoichiometry (n), as well as change in enthalpy and entropy (ΔH and ΔS) of the reaction. The resulting heat is plotted against the respective Drug/Protein molar ratio values in the Fig. 3.

The upper panel shows calorimetric response with consecutive addition of Nadolol into the sample cell filled with HSA. The bottom panel shows the plot of the amount of heat exchanged per injection as a function of the [Nadolol]/[HSA] mole ratio.

The upper panel of Fig. 3 shows the raw data for sequential injections of the Nadolol into HSA Protein solution and the lower panel shows negative heat deflection in the plot of the amount of heat liberated per injection as a function of the [Nadolol/[HSA] molar ratio. The association constant (Ka) and standard enthalpy change (ΔH^o) have been directly obtained after the best nonlinear least-squares fitting for the integrated heats by using single site binding model. [16]

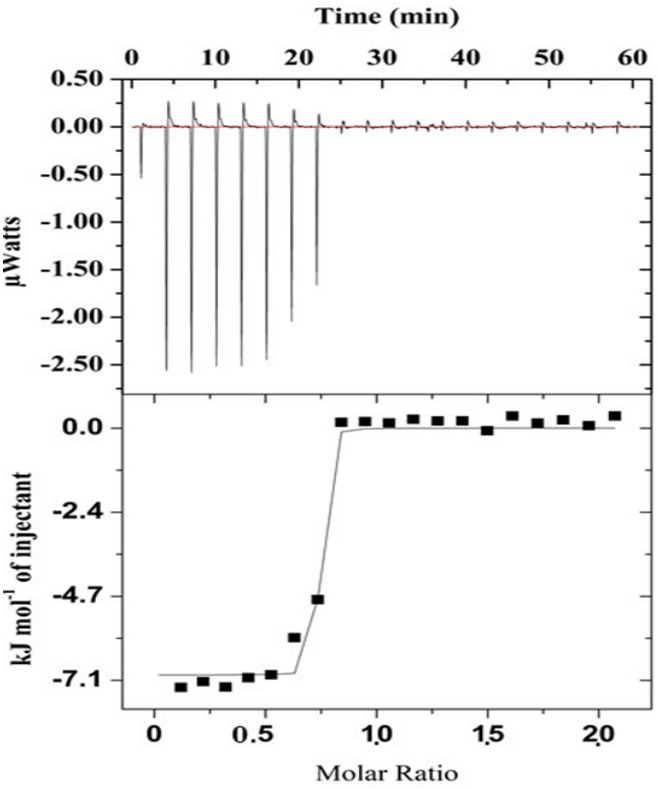

Fig. 3: Isothermal Calorimetric profile of HSA-Nadolol system

The calculated thermodynamic parameters, that include the binding constants (K_b), stoichiometry (N), enthalpy ($\Delta H°$) of binding, entropy ($\Delta S°$) of binding and free energy (ΔG^o) of binding are tabulated in Table 1.

Table 1: Thermodynamic parameters and association constant for the binding of Nadolol with HSA from ITC experiment at T = 298.15 K and in 10 mM phosphate buffer (pH 7.4)

System	$10^6 \cdot K_a$ /Lmol^{-1}	$\Delta H°$ /kJ·mol^{-1}	$T\Delta S°$ /kJ·mol^{-1}	$\Delta G°$ /kJ·mol^{-1}
HSA: Nadolol	8.73	-6.77	4.42	-92.74

The equilibrium constant value as obtained from the ITC measurement was K_a = 8.73 x 10^6 $Lmol^{-1}$ corresponding to a Gibbs free energy ΔG^o =−92.74 kJ/mol^{-1}, which indicate that reaction occurred spontaneously with favourable interaction for nadolol with HSA. The binding reaction had a favourable enthalpy, ΔH^o of about −6.77 kJ/mol. The global parameter enthalpy (ΔH^o), involves numerous contributions such as hydration effects, direct non-covalent interactions at the protein-ligand interface and conformational changes of the drug and proteins [17]. The $\Delta H < 0$ was associated with a favourable entropy contribution ($T\Delta S > 0$), as determined by the titration experiments. The entropic effect of the binding process is ΔS = 14.9 J/mol/K and bring an additional contribution to the negative Gibbs free energy. The negative enthalpy change $\Delta H < 0$ combined with the positive entropy change $\Delta S > 0$ further, confirm that both hydrophobic and electrostatic interactions contribute to the binding process [18]. Variations in calculated parameters from ITC measurements and fluorescence spectroscopy may be attributed to the fact that ITC measures a global change in the thermodynamic property, while fluorescence spectrescopy measures the local changes around the fluorophore (Trp-214) only [19].

3.2. Molecular docking study of HSA-Nadolol binding

Experimental results were analysed on the basis of docking score: higher the docking scores, higher the stability of the protein ligand complex which further provide basis for Nadolol/HSA interaction. Docking parameters setting and distance-dependent dielectric functions were two important parameters used in the calculation of the electrostatic and the van der Walls forces, respectively. For docking study, nadolol was docked in the subdomain IIA binding pocket of HSA to elucidate their interactions. Bulky nadolol drug molecule, get accommodated in the cavity near W214 residue thus making a favourable binding profile within this cavity. Different other residues involved in HSA-Nadolol interaction are shown in 2D plot (Fig. 3).

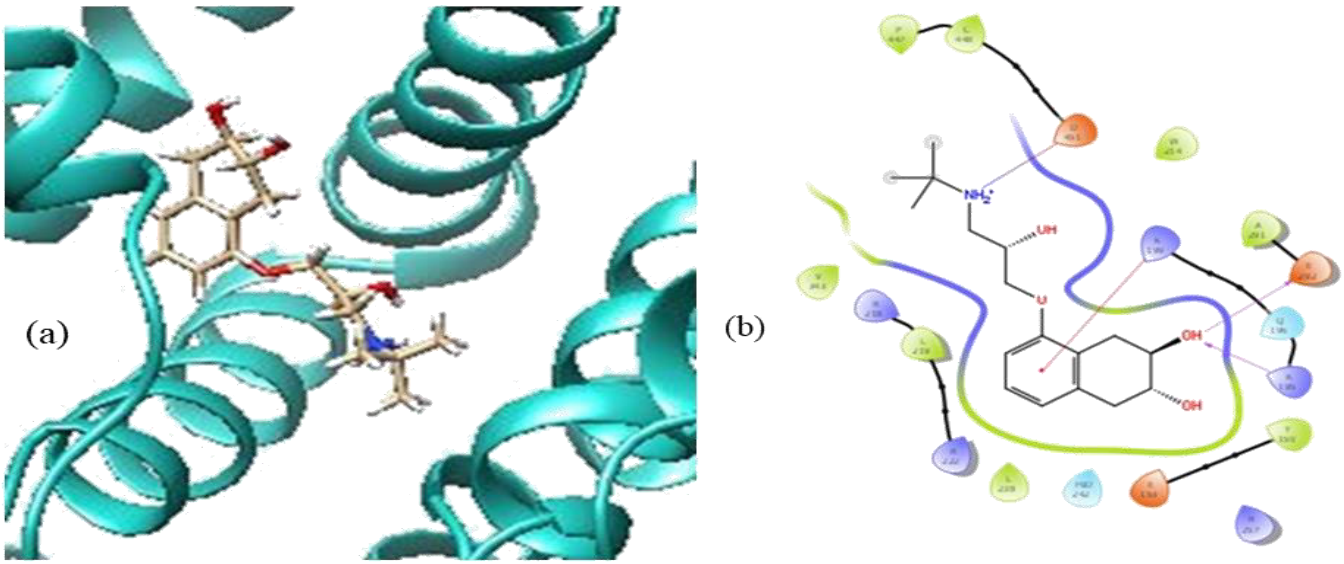

Fig. 4: (a) Docked complex of Nadolol and HSA in Sudlow's Site I; (b) 2D representation displaying types of interactions.

As shown in the Fig.4, there is a formation of complex between AH and HSA by forming salt bridge interactions with Asp451, hydrogen bonding in Lys 195, Glu 292 and cation-pi interactions with Lys199 coupled with other polar and non-polar interactions. Key players involved in the formation of stable complex of HSA-nadolol are only these residues.

4. CONCLUSION

In this research we have investigated the interaction of nadolol with HSA at the molecular level under physiological situation by employing isothermal titration calorimetric and molecular docking methods. The thermodynamic and molecular docking analysis reveals the interaction between nadolol and HSA molecule to

be governed by hydrogen bonding and cation-pi stacking interactions. This study will provide a deep insight in to the nadolol induced changes into the microenvironment of proteins that can be utilized towards for drug-development with enhanced efficacies and improved drug delivery.

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