Valproate effects on chemophysical properties of Human Serum Albumin

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ABSTRACT

Albumin has a fundamental role in human body. Its main tasks in blood are to regulate osmotic blood pressure, maintaining the pH, and transporting metabolites and drugs throughout the vascular system. Pharmacological studies of the interaction of drugs on HSA are important due to structural and functional changes of this vital protein; thus, here in this research the effect of valproate as a common drug for epilepsy disorders is evaluated in the presence of hexadecyl pyridinium bromide (HPB) as a positive surfactant in normal and fever condition. Electrochemical method was used to investigate the binding number of HPB molecules to HSA in the absence and presence of valproate by evaluating the concentration of free HPB in 37oC and 42oC temperatures. HSA affinity for valproate binding studied via ligand binding process for normal and fever temperatures. The findings indicate that, there is a significant difference in valproate binding to albumin at physiological and pathological temperatures. The consequences are the same in the presence of HPB; in other words, HSA binding tendency to HPB in the presence of valproate was totally altered because of HSA major conformational changes in fever condition. In conclusion, corrected dosage of valproate is needed for fever condition relative to normal temperature and the patients under prescription of different medications in fever condition should have different orders due to the interferences of drugs.

Keywords: Human serum albumin (HSA); Valproate; Hexadecylpyridinium bromide (HPB); Conformational changes.

INTRODUCTION

Albumin as the most dominant protein in human serum[1, 2] possess specific role in transporting metabolites and drugs in the vascular system[3, 4]. This protein is a single strand (MW = 67.5 kD) including 585 amino acid with abundance of 40 mg ml−1 in the blood plasma[5]. It has a high affinity binding sites for several endogenous and exogenous compounds such as metals such as Cu2+ and Zn2+, fatty acids, amino acids and metabolites and many drug materials[6-8] that pharmacological researches implies on the interaction of HSA and many drugs [9], which will be transported to other parts of the body[10]. This vital protein has been applied as a model protein for protein folding and ligand binding researches in recent years. In fact, may factors can possibly affect the structure and function of human serum albumin such as pH, temperature, and binding of different ligands [11]. Valproate (VPA) is an effective drug, which is used widely for treatment of different kinds of diseases such as seizure kinds both in adults and in children with epilepsy syndromes, acute bipolar depression, chronic disease, migraine ,and as adjuvant chemotherapy [12-14]. Hexadecyl pyridinium bromide (HPB) is a cationic surfactant including a positive head group and a hydrophobic tail [6]. Due to vita role of albumin in human body, evaluating the protein conformation alteration is essential for understanding protein stability and its effectiveness in medicine transferring[15]. Here in this research the effect of valproate in the presence of HPB on the conformation changes of HSA has been studied.

MATERIALS AND METHODS

Human serum albumin and reagents with analytical grade were purchased from Sigma and

Merck respectively. Phosphate buffer (10 mM, pH = 7.0) was used throughout the study. 0.2 mg/ml of albumin was prepared and used.

Three electrode cells including HPB, silver nitrate as reference and valproate were used for electro-chemical evaluations, equipped with a Teflon stopper with holes to hold electrodes in appropriate configurations for minimizing the solution resistance in the electrochemical study in this experiment. The binding interaction of HSA-HPB(Hexadecyl pyridinium bromide) in the presence of valproate at 37 oC and 42 oC was studied using a Fluke potentiometer. Electrochemical impedance measurements were performed at open circuit potential with proper redox potential as bias potential. Electrochemical signals were calibrated to HPB and valproate concentrations that were used in order to determine the concentrations of free HPB and valproate. HSA concentration was 0.2 mg/ml. Calibration was performed by adding 10, 20, 40, 80, 200, 700, and 1500 µl of HPB solution. Finally, the ratio for HPB binding to each mole of macromolecule was calculated[6]. UV absorbance of Albumin in 280 nm in phosphate buffer (10 mM, pH = 7.0) was provided by Unico UV spectrophotometer.

RESULTS

The binding isotherms of HSA-Valproate interaction in the 37oC and 42oC temperatures are shown in figure1. As depicted in figure 1, HSA affinity for Valproate binding in the both temperatures are different, so fever condition promotes more ligand binding.

Since HSA has a dual behavior with valproate in the range of temperatures, the effect of valproate on physicochemical properties of HSA investigated. To confirm temperature effect on HSA, absorbance of Albumin in 280 nm for temperature range of 35oC - 50oC is represented in figure 2.

HPB as a cationic surfactant is a proper probe for lyses of HSA structural aspects. In figure 3, HSA-HPB binding isotherms in the 37oC and 42oC are shown in figure 3.

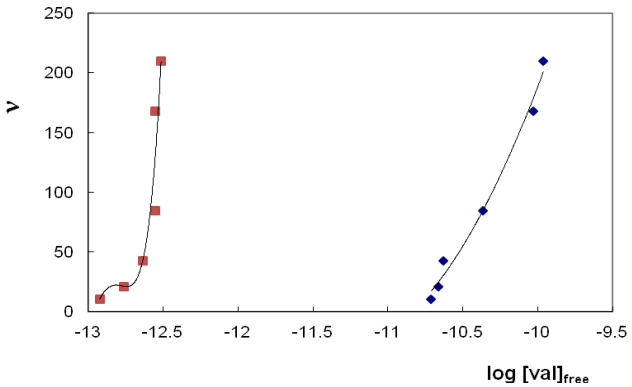

Figure1. Binding number of valproate molecule to HSA molecule increases as the concentration of this drug is enhanced (left 42oC and right 37oC).

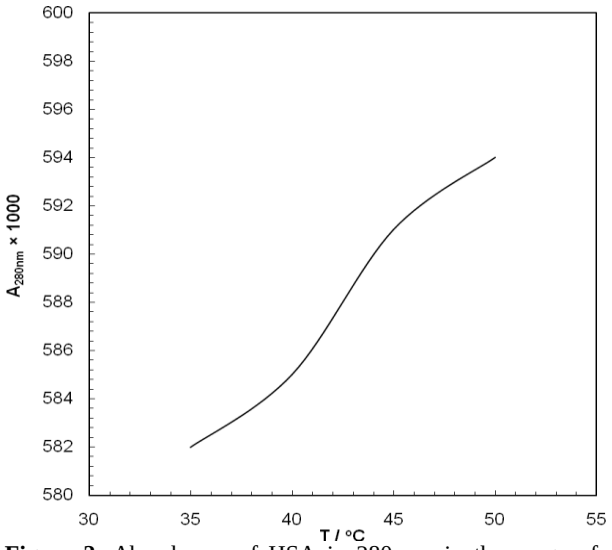

Figure 2. Absorbance of HSA in 280 nm in the range of 35oC-50oC temperatures.

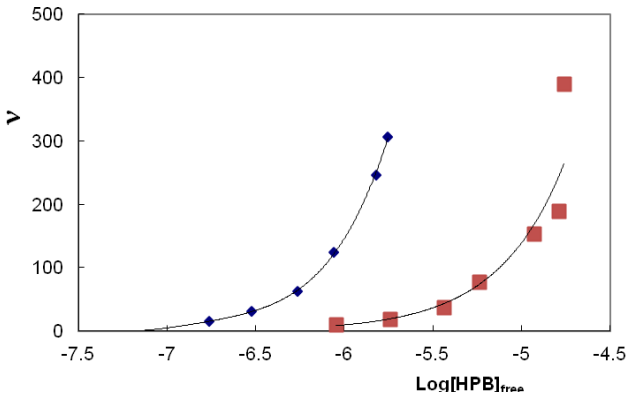

Figure3. Evaluation of HPB binding affinity to HSA (left 37oC and right 42oC).

Valproate may HPB-HSA interaction; therefore, isotherms of HPB-HSA interaction in the presence of therapeutic dose of valproate are illustrated in figure 4.

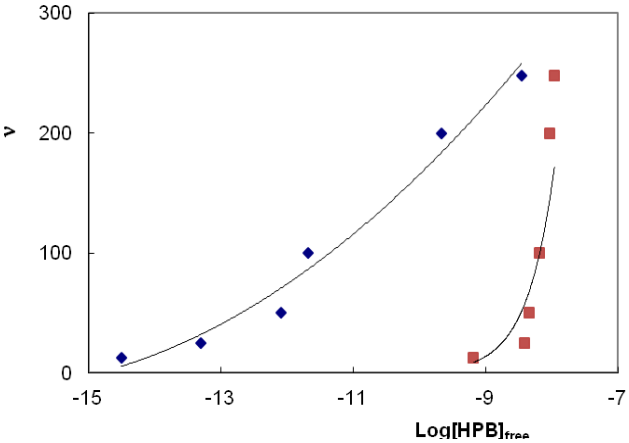

Figure4. Comparative evaluation of HPB binding affinity to HSA (left 37oC and right 42oC) in the presence of therapeutic dose of valproate.

DISSCUSSION

Temperature has important effects on the b inding properties of biomacromolecules[16]. In the literature, it is reported that changing of temperature from 37oC to 42oC, effects on the structural features of HSA[4]; as it is depicted in the figure 1, there is a specific difference in valproate binding affinity to HSA in 37oC and 42oC.

In fact, due to conformational changes in fever condition, HSA affinity for valproate is increased at 42oC relative to 37oC. In a way that HSA saturation of valproate at 42oC is corresponded to lower concentration of valproate in comparison with 37oC. In other words, it is reasonable that increasing temperature probably opens new binding sites for valproate.

This finding expresses that starting of ligand binding and saturation of the binding sites in the 37oC requires more concentrations of ligand. HSA beside the other functional role is a main drug carrier in the blood, it can be assumed that binding of some drugs can alter its function and structure. It is reported that interaction of acetaminophen with HSA induces conformational change on the protein[17]. Sodium valproate is a common drug that is used for epilepsy disease[18]. It can be concluded that, in the fever condition the bounded valproate is increased relative to free valproate due to the increment in HSA affinity for valproate; hence, it should be consider that therapeutic dose for valproate for physiologic and pathologic conditions are different. In figure 2, it is emphasis in the effect of temperature effect on HSA structural aspects. It is a reasonable to consider that binding of valproate may effects on the HSA structural aspects. Figure 3 shows the binding isotherms of HSA-HPB interactions in the absence of valproate in the 37oC and 42oC temperatures. In which HPB binds to HSA in dissimilar manners in different temperatures. As it is shown in figure3, fever condition reduces HSA affinity for HPB.

It is expected that, valproate changes HSA physicochemical properties. Consequently, HSA-HPB interaction was investigated in the presence of therapeutic dose of valproate. Since HPB is a cationic ligand and valproate is an anionic drug, it is possible that an electrostatic interaction occurs between them. For correction of this unfavorable effect, probable interaction of HPB-valproate was studied separately.

The results indicate that, these interactions occur in the very low concentration of HPB; therefore, they cannot influence on the binding isotherms HSA-HPB in the presence of valproate. Correspond to the binding isotherms of HSA-HPB interaction in the presence of valproate (see figure4), it seems that valproate increases HSA affinity for HPB binding. On the whole, these findings refer two proper effects; the first is the effect of valproate on the HSA structure in the 42oC that leads to the decrement of exposed binding site for HPB on the surface of the protein due to the mild structural change of HSA, and the second is a different manner of effectiveness of valproate on the HSA in the 42oC comparing with the 37oC, as temperature changes from 37oC to 42oC alters the structure of protein [7].

The binding process of HPB to HSA in physiological temperature occurs in the wide range of HPB concentration, but it takes place in narrow range for 42oC.

CONCLUSION

Temperature plays main role in the affinity of HSA-valproate binding process. HSA-valproate interaction alters binding properties of HSA that lead to the structural and functional alteration on the protein. This effect is a considerable process in the 42oC relative to 37 oC. The maximum value of human body temperature in the pathological condition (fever) is 42oC; consequently, it can be concluded that, the therapeutic dose of valproate should be corrected for this condition. Another significant finding in this research is, while the patients consume valproate with other medications, they should have a different dosage prescription for these medications due to probable interferences.

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