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In vitro binding comparison of cephalosporins to human serum albumin by spectroscopy and molecular docking approaches: A novel structural pursuing

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ABSTRACT

Contamination of antibiotic residues in food and water is an understudied safety concern and is increasingly gaining importance worldwide due to the rising rates of drug resistant organisms. As a class of antibiotics targeting β-lactam, cephalosporin is frequently used in treatment of bacterial infections. In this study, molecular modeling approaches in combination with absorbance, fluorescence and circular dichroism spectroscopy was employed to investigate the interactions of four cephalosporins (cefalexin, cefaclor, cefixime and cefepime) with human serum albumin (HSA). In the mechanism discussion, it was proved that the fluorescence quenching of HSA by cephalosporin is a result of the formation of cephalosporin-HSA complex. Binding parameters calculating from Stern-Volmer method and Scatchard method showed that cephalosporin bind to HSA with the binding affinities of the order 10^3–10^4 L·mol^{-1}. The thermodynamic parameters studies revealed that the binding was characterized by negative enthalpy and negative entropy changes and the hydrogen bonds and van der Waals interactions may play major role in complex formation. Furthermore, the investigated result indicates that azyl served as a hydrophilic group to change the chemical property for drug, the chlorine atom on the R_1-group of cefaclor weakened the overall molecular polarity or hydrophilicity, while the binding site on HSA was determined by the carboxylate of cefepime. Some other structural characteristics for four kind of cephalosporins were also exhibited in this paper.

1. Introduction

The widespread use of antibiotics to reduce bacterial growth generally and to treat infections specifically has resulted in contamination of antibiotic residues in food which has triggered global attention on food safety issue. For example, antibiotics are widely used in the livestock breeding industry to defense the disease emergence or disease spread, which are motivate the survey for antimicrobial residues in edible food largely [1,2]. The contamination of milk with antibiotic residues can cause serious health effects in humans [3]. The drug resistance of even non-typhoidal *Salmonella* isolates has also been found during surveys of egg layer flocks and egg shells [4]. Compared with other antibiotics such as aminoglycosides and polymyxins, cephalosporins has superior antibiotic activity, efficiently destroying β-lactam rings and reduced nephrotoxic effects [5,6]. The mechanism of antibacterial activity for the expanded-spectrum cephalosporin compounds is through inhibition of cell wall synthesis. 7-aminocephalosporanic acid (7-ACA) synthesized from the cephalosporin C with new groups incorporated in the side-chains at positions 3 and 7, is an intermediate of the effective treatment of bacterial infection [7,8]. As the first-generation cephalosporin antibiotic, cefalexin was used to treat urinary tract infections, respiratory tract infections, skin and soft tissue infections because of its effective antibacterial activity against both gram positive and negative organisms [9,10]. Cefaclor, a second-generation cephalosporin antibiotic, has a structure resembling cefalexin within chlorine of 3 position. Similarly, cefaclor is not only active against the most prevalent Gram-positive and Gram-negative pathogens, but also well tolerated for the treatment of respiratory tract infections [11,12]. Cefixime is a broad-spectrum oral cephalosporin that is effective, inexpensive and secure profile, especially for the treatment of pharyngitis [13]. However, the high-level use of cefixime may be related to the recent gonorrhea treatment failures [14]. Cefepime is a parenteral fourth-generation cephalosporin antibiotic with an extended spectrum of antimicrobial activity, frequently administered to treat sepsis and pneumonia in critically ill patients and post-operative

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patients [15,16]. The clearance of cefepime occurs mainly through renal function, and this has been shown to cause renal toxicity with high doses cefepime in patients with compromised renal function [17].

Cefalexin, cefaclor, cefixime and cefepime mediate similar effects through different side-chains. Contrastive analyses of the structural diversity are unquestionably beneficial to comprehensive pharmaceutical developmental approaches. Human serum albumin (HSA), the most abundant transport protein in blood plasma, has the ability to bind and deliver diverse exogenous and some endogenous ligands [18,19]. In recent years, HSA has attracted much attention with respect to its effect on the pharmacokinetic behavior, physicochemical properties and physiological functions of different drugs [20–22]. In our study, investigating the functional drug carrier HSA and its interaction with four kinds of cephalosporins can help define the functionality of these structural variations. The quenching mechanisms, binding modes, and conformational variations and conduct a thermodynamic analysis of binding have been studied in this paper. All experiments were based on the structural perspective and provided a theoretical structural forecast for design of new inhibitors at the molecular level.

2. Materials and methods

2.1. Materials

HSA and hydroxymethyl aminomethane (Tris) were obtained from Sigma-Aldrich (St. Louis, MO, USA); cefalexin, cefaclor, cefixime and cefepime were purchased from Tianjin science and Technology Co., Ltd. (Tianjin, China); ibuprofen was obtained from Hubei Biocause Pharmaceutical Co., Ltd. (Hubei, China; the purity no less than 99.7%); warfarin was purchased from Trust Chemical Industry Co., Ltd. (Nanjing, China; the purity no less than 99.5%); NaCl, HCl, and other standard chemicals were all of analytical grade. HSA was dissolved in Tris-HCl buffer solution (0.05 $mol \cdot L^{-1}$ Tris, 0.15 $mol \cdot L^{-1}$ NaCl, pH 7.4) and was kept in the refrigerator at 0–4 °C. Stock solution of high concentration of cefaclor, cefixime and cefepime were dissolved in dimethyl sulfoxide, and cefalexin was dissolved in ultrapure water. Appropriate blanks, run under the same conditions, were subtracted from the sample spectra.

2.2. Measurement of spectrum

Absorption spectra measurements were carried out using a UV-9000S spectrophotometer (Yuanxi, Shanghai, China) equipped with a 1.0 cm quartz cell. The wavelength range was 190–500 nm with slit width of 2.0 nm.

Fluorescence measurements were conducted using LS-55 spectrophotometer (PerkinElmer, American) equipped with a 1.0 cm quartz cell and a thermostat bath. An excitation wavelength of 295 nm was chosen since it provides no excitation of tyrosine residues and therefore neither emission nor energy transfer to the lone indole side chain would be nonnegligible.

The emission and excitation slit widths used throughout the experiment were 10 nm and 5 nm while the scanning rate was 500 $nm \cdot min^{-1}$. Synchronous fluorescence spectra was recorded at 298 K in the wavelength range of 300 − 380 nm at the fixed intervals of $\Delta\lambda = 60$ nm. For three-dimensional fluorescence spectroscopy, the emission wavelength was recorded at 220–500 nm and the excitation wavelength was recorded at 200–350 nm.

Circular dichroism (CD) spectra were measured with a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature over a wavelength range of 320–200 nm and under constant nitrogen. Quartz cells having a path length of 1.0 cm were used at a scanning speed of 50 nm/min.

2.3. Molecular modeling

Molecular docking experiments were performed using the docking software Surflex-Dock Sybyl-X 2.1.1. The native structure of HSA was obtained from RCSB Protein Database Bank (PDB ID: 1H9Z), the structure of the biopolymer was analyzed and prepared for the docking experiment [23]. Hydrogen atoms with H-bond orientation were added. The biopolymer was surface charge determined using AMBER7 FF99 method and the drug molecule was charge distribution determined with Gasteiger-Huckel method [24]. A Tripos Force Field was used to optimize the molecular conformation of cephalosporins, using the ligand model to generate the protocol, and all the water molecules were removed before analysis. Other parameters used in the docking program were determined through iterative processes.

3. Results and discussion

3.1. Structural analysis and characteristic spectra of cephalosporins with HSA

The structures of four kinds of cephalosporin antibiotics are shown in Fig. 1, and the different side-chains at positions 3 or 7 of 7-ACA are listed in Table 1. It was found that the structural differences between cefalexin and cefaclor merely reflect a difference in the R_1-group, replacing a methyl group with chloride ion. Cefixime and cefepime have similar molecular structures except for the interlinked side-chains at the positions 3 and 7 of 7-ACA which due to the quite distinct vinyl electropositive R_1-group and the deprived carboxylate radical of the R_2-group of cefepime. The substituent group at position 4 of 7-ACA doesn't contain a hydrogen atom causing an increase in electronegativity, defining an additional variation between cefepime and the other compounds in molecular structure.

UV–vis absorption and fluorescence spectroscopy are widely used in the analysis of molecular structure and drug-protein interaction [25,26]. Fig. 2 shows the UV–vis absorption spectra of HSA with various concentrations of antibiotics, and the inset of Fig. 2 shows the effect on the fluorescence intensity of antibiotics when bound to HSA. All curves labelled '*a*' represent the spectra of HSA alone and the concentration used for absorption or fluorescence measurement equals to 1×10^{-5} $mol \cdot L^{-1}$ or 5×10^{-6} $mol \cdot L^{-1}$ respectively. The absorbance peak of HSA at 278 nm varied in amplitude with increasing concentrations of antibiotic. And the absorbance peak increasingly blue-shifted as the concentration of cefalexin increased (Fig. 2 (A)). This phenomenon may be due to B absorption band of π—π^* transition of benzene in R_2- group. However, increasing concentrations of cefaclor did not alter the absorbance peak of HSA (Fig. 2 (B)) significantly. Since the only structural difference between cefalexin and cefaclor is merely the substitution of a methyl group with a chloride ion, it can be concluded that the difference of absorbance spectra is caused by this variation. Large numbers of electrons are transferred from the electron-donating chlorine atom to the adjacent group, which may cause the differences in absorbance peaks of the HSA-cefalexin complexes with HSA-cefaclor complexes. Different to cefalexin and cefaclor, addition of cefixime induced a non-ignorable red-shift (Fig. 2 (C)). The connective R_1-group is an unsaturation vinyl, which could participate in the formation of conjugated structures with adjacent double bonds. The R_2-group of cefixime is attached with an unsaturated link forming the conjugated structures. The conjugative effect can occur in different chromophores, therefore, cause the original absorbent band of chromophore disappear and is being replaced by an absorbent band at longer wavelength and more intensive absorbance. Unfortunately, the absorption spectra of HSA by the interaction with cefepime did not show a red-shift (Fig. 2 (D)) despite the R_2-group of cefepime also having conjugated unsaturated double bonds. The difference between HSA-cefixime and HSA-cefepime may be caused by the difference between conjugations with a stable vinyl compared to the electropositive group of the R_1-groups. The electropositive R_1-