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Reactive oxygen species generation and human serum albumin damage induced by the combined effects of ultrasonic irradiation and brilliant cresyl blue

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ABSTRACT

In this paper, brilliant cresyl blue (BCB) was selected as a sonosensitizer. The sonodynamic damage to human serum albumin (HSA) in the presence of BCB and the mechanism were studied by means of fluorescence and absorption spectra. Firstly, BCB could quench the intrinsic fluorescence of HSA obviously and the quenching mechanism was static quenching due to the formation of HSA-BCB complex. The results of the displacement experiments and the molecular modeling suggested that the binding site of BCB on HSA was site I, and hydrophobic forces and hydrogen bonds played major roles in the interaction between HSA and BCB. Secondly, the damage of HSA induced by the combined effects of ultrasonic irradiation and BCB was more efficient than that only BCB or ultrasound irradiation, which confirmed that BCB had sonodynamic activity. The damage degree of HSA was positively correlated with reactive oxygen species (ROS) produced in the system, which indicated that ultrasound could activate BCB to produce ROS, and the kinds of ROS produced by the combined effects of ultrasonic irradiation and BCB were mainly hydroxyl free radical, singlet oxygen and superoxide anion radical.

1. Introduction

Sonodynamic therapy (SDT) is a potential treatment modality for many solid tumors which relies on the combined effects of ultrasonic irradiation and sonosensitizer [1]. Based on the focusing and strong penetrating ability of ultrasound to biological tissue, the sonosensitizer enriched at the tumor site can be activated by ultrasonic irradiation to produce reactive oxygen species (ROS), and thereby generate antitumor effect [2]. As the local cytotoxic effect of this method can reduce the damage of normal tissue around the tumor site to the minimum and be non-traumatic, it has shown unique advantages in non-invasive treatment of deep tumors. Therefore, SDT has attracted extensive attention since it was proposed [3]. The development of sonosensitizer with good sonodynamic activity is one of the main research contents of SDT, as well as the key to promote the application of SDT in clinic [4].

At present, two methods are mainly used to screen and study the sonodynamic activity of the compounds. The first method is to study the combined effects of ultrasonic irradiation and the compound on the survival rate of tumor cells in vitro to confirm the sonodynamic activity of the compound. For example, Umemura et al. [5] studied the sonodynamic activity of rose bengal (RB) by taking S180 cells as the research model. The results showed that the damage effect of S180 cells induced by ultrasound was enhanced two to three times with 160 μmol/L RB, while no cells damage was observed with RB alone, suggesting that RB had good sonodynamic activity. The second method is to study the damage of biomacromolecules (protein or DNA) caused by the combined effects of ultrasonic irradiation and the compound in vitro by spectral methods to confirm the sonodynamic activity of the compound. For example, Wang et al. [6] studied the damage of DNA by the combined effects of ultrasonic irradiation and hematoporphyrin gallium (Hp-Ga) taking DNA as the model of study by spectral method. He et al. [7] used bovine serum albumin (BSA) as the research model to confirm the sonodynamic activity of promethazine hydrochloride (PMT). Damage of substances (protein or DNA) in tumor cells is one of the reasons leading to abnormal apoptosis of tumor cells. Existing research results have confirmed that proteins and DNA in tumor cells are important intracellular targets of SDT [8,9], which provides strong theoretical support for this method. So far, many compounds such as PMT [7], methylene blue [10], toluidine blue [11], dioxopromethazine hydrochloride [12], berberine hydrochloride [13], riboflavin [14] and fluorescein derivatives [15] have been proved that they have good sonodynamic activity using the second method.

Brilliant cresyl blue (BCB) is a quinine imide dye with planar structure [16]. So far, a variety of dibenzo-heterocyclic compounds such as acridine orange [17], oxanthracene [18–20] and phenothiazine

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compounds [7,10–12] have been proved good sonodynamic activity, but the sonodynamic activity of BCB has not been reported. Herein, human serum albumin (HSA) and BCB were selected as a model protein and sonosensitizer, respectively. Firstly, the binding characteristics of BCB to HSA were studied by fluorescence spectrometry and molecular docking method. Secondly, the damage of HSA and the generation of ROS induced by the combined effects of ultrasonic irradiation and BCB were studied by fluorescence spectrometry and oxidation-extraction spectrophotometric method. It is expected that the results of this research will some provide valuable information for the development of sonosensitizer with good sonodynamic activity for the application of SDT.

2. Experimental sections

2.1. Chemicals and reagents

HSA (Fraction V, purity, 96–99%) was purchased from Beijing Solarbio Science & Technology Co., Ltd. China. BCB was purchased from Sinopharm Chemical Reagent Co., Ltd. China. The stock solutions of HSA and BCB were prepared by the 0.05 mol/L NaCl-Tris-HCl buffer solution (pH 7.40). Warfarin (purity, 98%) was purchased from Tianjin Elong Co., Ltd. China. Ibuprofen (purity, 99.5%) was purchased from Zhengzhou Debao Fine Chemical Co., Ltd. China. The stock solutions of warfarin and ibuprofen were first dissolved in small amounts of ethanol, and then prepared by the 0.05 mol/L NaCl-Tris-HCl buffer solution (pH 7.40). 1,5-Diphenylcarbohydrazide (DPCI) was purchased from Sinopharm Chemical Reagent Co., Ltd. China. The stock solution of DPCI was first dissolved in small amounts of acetic acid, and then prepared by the 0.05 mol/L NaCl-Tris-HCl buffer solution (pH 7.40). The other reagents used in the experiments all were of analytical grade.

2.2. Spectra measurements

The fluorescence emission spectra were measured on an F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Japan) equipped with a 150 W xenon lamp. The experimental temperature was controlled by an SC-15 thermostat bath (Shanghai Bilon Instrument Co., Ltd., China). In the fluorescence quenching experiment, the fluorescence emission spectra were obtained with constant HSA concentration at 1.0×10^{-5} mol/L and the BCB concentrations ranging from 0.0 to 5.0×10^{-5} mol/L at 0.5×10^{-5} mol/L intervals. The fluorescence emission spectra at 290, 300 and 310 K were measured from 290 to 450 nm at an excitation wavelength of 280 nm. In the displacement experiment, the fluorescence emission spectra were obtained with constant HSA and BCB concentration at 1.0×10^{-5} mol/L and 2.5×10^{-5} mol/L, respectively, and the warfarin (or ibuprofen) concentrations varying from 0.0 to 8.0×10^{-5} mol/L at 1.0×10^{-5} mol/L intervals. The fluorescence emission spectra at 290 K were measured from 290 to 450 nm at an excitation wavelength of 280 nm. The excitation and emission slits were both 5.0 nm. The scanning rate was set at 1200 nm/min. Furthermore, the fluorescence intensity data were corrected to subtract the inner filter effect by the Eq. (1) [21,22]. In Eq. (1), F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are the absorbance of HSA-BCB system at the excitation and emission wavelength, respectively. The absorption spectra were measured on a UV-2550 spectrophotometer (Shimadzu Co., Japan).

$$F_{cor} = F_{obs} \times e^{\frac{A_{ex}+A_{em}}{2}} \quad (1)$$

2.3. Molecular docking method

Molegro Virtual Docker 2008 (v. 3.0) program was used to perform the molecular docking between HSA and BCB. The structure of BCB was drawn by ChemWindow 6.0 program. The crystal structure of HSA (PDB code 1GNJ [23]) was obtained from the Research Collaboratory for Structural Bioinformatics. After the original ligands were removed, BCB was docked into the 3D structure of HSA. The water and amino acid molecules having no interaction with BCB were removed. The molecular docking process is the same as what we previously reported [24].

2.4. Sonodynamic damage to HSA in the presence of BCB

Firstly, the influence of BCB concentration on sonodynamic damage to HSA was examined. The final DPCI concentration was kept at 1.0×10^{-5} mol/L. The BCB concentration was changed from 0.0 to 2.5×10^{-5} mol/L. After ultrasonic irradiation (the schematic diagram of ultrasonic apparatus is shown in Fig. 1) 3.0 h, the fluorescence emission spectra of the sample solutions were measured by the fluorescence spectrophotometer. Secondly, the influence of ultrasonic irradiation time on sonodynamic damage to HSA was investigated. The final concentrations of HSA and BCB were both kept at 1.0×10^{-5} mol/L. The ultrasonic irradiation time was changed from 1.0 h to 5.0 h. The output power of the ultrasonic apparatus is 200 W and the fixed frequency is 40 kHz. The system temperature was kept at 298 ± 1 K by a circulation constant temperature water bath.

2.5. ROS detection

The experiments of ROS detection were performed according to the method previously reported [12]. Firstly, the influence of BCB concentration on ROS generation was examined. The final DPCI concentration was kept at 2.5×10^{-3} mol/L. The BCB concentration was changed from 0.0 to 2.5×10^{-5} mol/L. After ultrasonic irradiation 3.0 h, the sample solutions (10 mL) were extracted with 10 mL benzene carbon tetrachloride (1:1) mixed solution and detected the absorbance at 563 nm by spectrophotometer. Secondly, the influence of ultrasonic irradiation time on ROS generation was examined. The final concentrations of DPCI and BCB were kept at 2.5×10^{-3} mol/L and 1.0×10^{-5} mol/L, respectively. The ultrasonic irradiation time was changed from 1.0 h to 5.0 h. Thirdly, the influence of ROS scavenger on ROS generation was examined. The final concentrations of DPCI and BCB were kept at 2.5×10^{-3} mol/L and 1.0×10^{-5} mol/L, respectively. The final concentrations of D-Mannitol (D-Man), L-Histidine (L-His), p-Benzoquinone (PBQ) and Vitamin C (V_C) were all 2.0×10^{-2} mol/L. The ultrasonic irradiation time was 3.0 h.

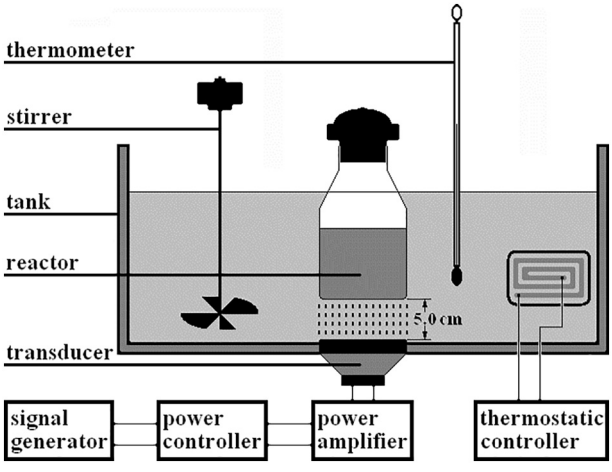

Fig. 1. Schematic diagram of ultrasonic apparatus.