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RESEARCH PAPER

Interaction between Copper, Zinc Superoxide Dismutase and Quinolones

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Abstract: The interactions between quinolones (norfloxacin, ciprofloxacin, enoxacine) and copper, zinc superoxide dismutase (CuZnSOD) were investigated using absorption and fluorescence spectra. Quinolones can make CuZnSOD fluorescence quenching. The quenching mechanism is static quenching. The binding constants of quinolones with CuZnSOD were obtained at various temperatures. According to the Förster nonradiative energy transfer theory, the distance of donors and acceptors can be obtained. The main acting force between them is electrostatic gravitation. Quinolones can decrease the activity of CuZnSOD, and the activity of CuZnSOD in rabbits blood being injected with ciprofloxacin also decreased obviously. The results provide some references for clinical diagnosis using the activity level of CuZnSOD, which varies in patients with different diseases.

Key Words: Quinolones; Copper, zinc superoxide dismutase; Spectrofluorimetry

1 Introduction

Norfloxacin (NF), enoxacine (ENX), and ciprofloxacin (CIP) are all good antibacterial medicines[1]. Drugs bring the action via the store and transport of human serum albumin. Therefore, it is important to study the interactions between drugs and human serum albumin[2–6]. However, there is a few of investigation of the reaction between drugs and copper zinc superoxide dismutase (CuZnSOD)[7]. CuZnSOD is a type of Superoxide dismutase (SOD, EC 1.15.1.1), which catalyzes the dismutation of the superoxide anion (O^{2-}) into hydrogen peroxide and molecular oxygen. CuZnSOD is one of the most important metalloenzymes in the first line of defense against oxidative stress. CuZnSOD counteracts the effects of these oxidizing substances and is involved in the pathophysiology of human diseases such as atherosclerosis, rheumatoid arthritis, and some tumors[8,9].

In this study, the interactions between NF, ENX, CIP, and CuZnSOD, the binding distance between the acceptor and donor, analysis of the binding mechanism of CuZnSOD and the drugs, and calculation of the binding constants, and the number of binding points were reported. The decrease of the activity of CuZnSOD caused by quinolones outside the body was also studied. The activity of CuZnSOD in the whole blood of rabbits decreased after injecting ciprofloxacin and recovering its initial level after the injection was stopped. Because the level of the activity of CuZnSOD varies in different patients with different disease, the result provides useful clinical information about the compatibility and use of drugs via its reaction at the molecule level.

2 Experimental

2.1 Reagents and instruments

The preparation of CuZnSOD from garlic accords with the method of literature[10,11], and then the enzyme was purified by DE-32 and G-25 pillars in series after dissolving in 10 ml of 0.20 M KH_2PO_4-K_2HPO_4 buffer solution (pH 7.60). The active effluents were collected, concentrated and dialyzed, frozen, and dried. The pure extract of CuZnSOD was stored in a refrigerator. 7.72×10^{-5} M CuZnSOD working solution was prepared when used.

A stock enoxacine 7.72×10^{-4} M (ENX, Biological Product

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Institution of Chinese Medicine) solution was directly dissolved in doubly distilled demineralized water. The working standard solution 7.72×10^{-5} M was freshly prepared by appropriate dilution with doubly distilled demineralized water. NF and CIP were prepared by the same procedure as ENX.

All the stocking solution and working solution should be preserved at 0–4 °C. 0.05 M pyrogallol working solution collocated by hydrochloric acid (0.10 M). 0.10 M Tris-HCl buffer solution of pH 7.40. All reagents were of analytical reagent grade and double distilled water was used throughout the experiment.

New Zealand white rabbits were bought from Shandong Agricultural Science Academy.

All fluorescent measurements were carried out on an RF-540 recording spectrofluorimeter (Shimadzu, Japan) equipped with xenon lamp source and an instrument of the constant temperature. A UV-265 recording spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells was used for the UV spectrum scanning. All pH values were measured using a pHS-3B digital pH-meter (shanghai Leici Device Works, China).

2.2 Experimental procedures

Solutions were added to 10 ml color comparison tubes in the following order: 2.00 ml Tris-HCl buffer solution, 1.00 ml 7.72×10^{-5} M CuZnSOD solution, and various volumes of 7.72×10^{-5} M ENX solution. The mixture was diluted to the mark with double distilled demineralized water. Fluorescence spectra were measured after 10 min. Using the same method, the fluorescence spectra of NF, CIP and CuZnSOD were obtained. The fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ 281 nm/345 nm.

Four rabbits (two male and two female, each weighing 1.5 kg) were separated into two groups at random including one male and one female. Each rabbit in experimental group was injected with ciprofloxacin every 24 h for seven days. One milliliter of blood was phlebotomized from a live rabbit 24 h after injection with ciprofloxacin. The activity of the CuZnSOD assays was carried out by the pyrogallol autooxidation method[12] , and the same procedure was carried out for the comparison group.

3 Results and discussion

3.1 Fluorescence quenching spectra

The fluorescence quenching spectra of CuZnSOD with varying concentrations of ENX is shown in Fig.1. It can be seen that the endogenesis fluorescence intensity of CuZnSOD at 345 nm was decreased regularly with the increase of concentrations of ENX. It indicates that the interaction and the energy transfer between CuZnSOD and ENX have occurred.

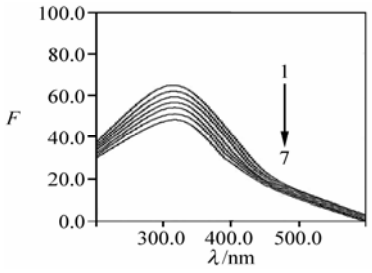

Fig.1 Effect of ENX on fluorescence spectra of CuZnSOD
[CuZnSOD] = 7.72×10^{-5} M, [ENX] = 7.72×10^{-5} M. $V_{CuZnSOD}$ = 1.00 ml. Different volumes of ENX were used: 1–7: 0.00, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 ml. The total volume of each is 10 ml in pH 7.40 Tris-HCl buffer solution, at 20 °C, $\lambda_{ex}/\lambda_{em}$ = 281 nm/345 nm

The fluorescence quenching spectra of CuZnSOD and CIP and NF have the same phenomena and are omitted here.

3.2 Fluorescence quenching mechanism

For illustrating its quenching mechanism further, the Stern-Volmer[3–6] graph of CuZnSOD and NF at various temperatures are shown in Fig.2.

As shown in Fig.2, it can be seen that the curves have favorable linear relationships, and the slopes of the quenching curves decreased with the increasing of temperature. It indicates that the static quenching interaction occurred between NF and CuZnSOD. To confirm the view, the procedure was assumed to be dynamic quenching. The quenching equation in this case is defined as follows[3–6,13]:

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

According to the Equation (1), the quenching constants and linear correlative coefficients for three types of drugs were shown in Table 1.

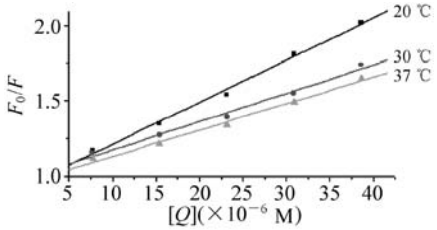

Fig.2 Stern-Volmer curves of NF and CuZnSOD at different temperatures

Table 1 Quenching constants and linear correlative coefficients for ENX, NF and CIP

Drugs	Temperature (°C)	Quenching rate constant (K_q) ($\times 10^{12}$ M^{-1} s^{-1})	Linear correlative coefficient (r)
ENX	20	6.19	0.9875
	30	4.94	0.9848
	37	4.50	0.9889
CIP	20	2.39	0.9959
	30	2.13	0.9917
	37	1.61	0.9961
NF	20	2.77	0.9936
	30	1.96	0.9862
	37	1.78	0.9963