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Study of the interaction between terazosin and serum albumin Synchronous fluorescence determination of terazosin

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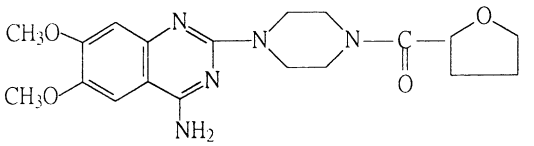
Abstract

The interactions between terazosin and bovine serum albumin (BSA) were studied by spectrofluorimetry. The binding constants of terazosin with BSA were measured at different temperatures. The effects of various metal ions on the binding constants of terazosin with BSA were also studied. The optimum conditions of synchronous fluorometric determination of terazosin were studied and the method was successfully applied to the determination of terazosin added to serum and urine samples (3σ detection limit 0.21 mg l^{-1}). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Terazosin; Bovine serum albumin; Synchronous fluorescence; Determination

1. Introduction

Terazosin is a new type of selectivity retarder of α_1 acceptors and its function in decreasing hypertension is similar to that of pazosin. But the effect of terazosin lasts much longer than pazosin and its adverse-effect is less [1]. However, if the patients take it above the normal dose, they will feel headache, dizziness, fatigue and so on. So, it is important to study the determination of terazosin and the reaction between terazosin and serum albumin. Various instrumental methods [2–4] have been reported for the determination of terazosin. In this study, we investigated the binding reaction and the effect of energy transfer between terazosin and bovine serum albumin (BSA), as well as the effect of temperature and metal ions by spectrofluorimetry. Moreover, the synchronous fluorescence technique was successfully applied to determine terazosin in blood serum and urine samples under physiological conditions.



Terazosin

1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl) carbonyl]

piperazine

2. Experimental

2.1. Apparatus

All fluorescence measurements were carried out on an RF-540 recording spectrofluorimeter (Shimadzu, Kyoto, Japan) equipped with a xenon lamp source and 1.0 cm quartz cells and a thermostat bath. A

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UV-265 recording spectrophotometer (Shimadzu) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum. All pH measurements were made with a pHS-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass–calomel electrode.

2.2. Reagents

BSA (Huamei Biotechnological, Beijing) and terazosin (Medicine Research Institution, Shandong, China) were used. 1.00×10^{-4} mol l^{-1}solutions of various metal ions, 0.2 mol l^{-1} Tris–HCl buffer of pH 7.4 and 0.5 mol l^{-1} sodium chloride were prepared. All reagents were of analytical reagent grade and double distilled water was used throughout.

2.3. Procedures

To a 10 ml comparison tube, 2 ml of sodium chloride solution, 1 ml Tris–HCl buffer, 0.5 ml of BSA solution and various amount of terazosin were added and were diluted to 10 ml with water and then were shaken. Fluorescence quenching spectra, absorption spectra and synchronous fluorescence spectra were obtained. Fluorescence quenching spectra were obtained at excitation and emission wavelengths of 286 and 300–500 nm, respectively and the range of synchronous scanning was: λ_{ex}: 340–430 nm, λ_{em}: 360–450 nm.

In addition, in the presence of metal ions, other fluorescence spectra could be obtained.

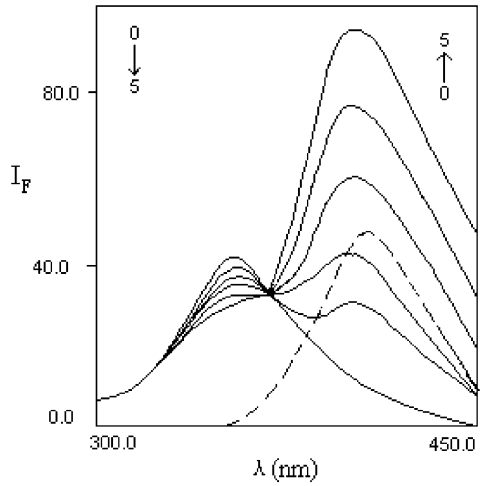

Fig. 1. Effect of terazosin on fluorescence spectra of BSA (λ_{ex} = 286 nm). (—): $C_{BSA} = 5.0 \times 10^{-6}$ mol l^{-1}; $C_{terazosin}$(0): 0, (1): 1×10^{-6}, (2): 2×10^{-6}, (3): 3×10^{-6}, (4): 4×10^{-6}, (5): 5×10^{-6} mol l^{-1}. (---): $C_{BSA} = 0$ mol l^{-1}; $C_{terazosin} = 2.0 \times 10^{-6}$ mol l^{-1}.

3. Result and discussion

3.1. Binding constant of BSA and terazosin

The fluorescence quenching spectra of BSA at various concentrations of terazosin are shown in Fig. 1. It is interpreted that the compound formed between terazosin and BSA quenched the fluorescence of BSA.

The Stern–Volmer and Lineweaver–Burk graphs [5] are shown in Fig. 2, at various temperatures. Fig. 2a

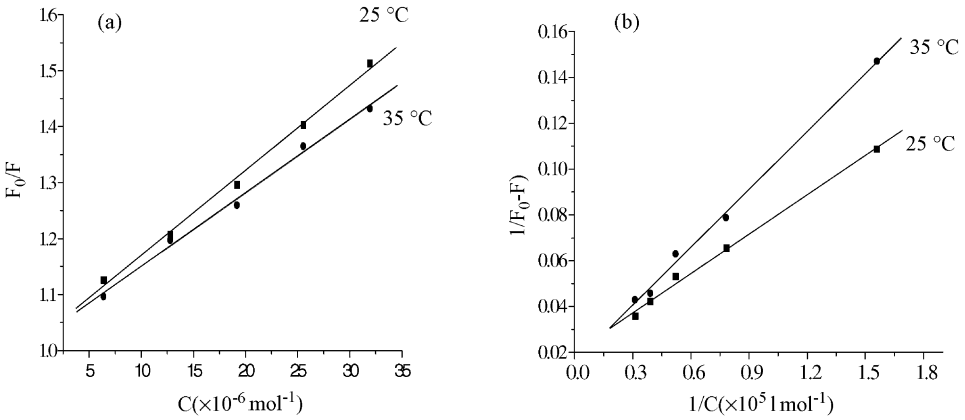

Fig. 2. (a): Stern–Volmer curves; (b) Lineweaver–Burk curves.

shows that plots are linear and the slopes decrease with increasing temperature. This indicates the static quenching interaction between terazosin and BSA. In order to confirm this view, the procedure was assumed to be dynamic quenching. The quenching equation is [5]:

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

where K_q, K_{sv}, τ_0 and [Q] are the quenching rate constant of the bimolecule, the dynamic quenching constant, the average lifetime of molecule without quencher and concentration of quencher, respectively. $K_{sv} = K_q\tau_0$ and $K_q = K_{sv}/\tau_0$. Because the fluorescence lifetime of the biopolymer is 10^{-8} s [6], the quenching constant (K_q: l mol^{-1} s^{-1}) can be obtained from the slope ($t = 25\,^\circ$C, $K_q = 1.52 \times 10^{12}$, $r = 0.9977$; $t = 35\,^\circ$C, $K_q = 1.37 \times 10^{12}$, $r = 0.9888$). However, the maximum scatter collision quenching constant of various quenchers with the biopolymer is 2.0×10^{10} l mol^{-1} s^{-1} [7]. Obviously, the rate constant of protein quenching procedure initiated by terazosin is greater than the K_q of the scatter procedure. So, this shows that the quenching is not initiated by dynamic collision but from compound formation. The static quenching equation is [5]:

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{K_D}{F_0[Q]} \quad (2)$$

The formation constant (K_A) can be obtained from Fig. 2b ($K_A = 1/K_D$, $t = 25\,^\circ$C, $K_A = 2.13 \times 10^4$, $r = 0.9976$; $t = 35\,^\circ$C, $K_A = 1.69 \times 10^4$, $r = 0.9959$). It shows that the binding constant between terazosin and BSA is great and the effect of temperature is small. Thus, terazosin can be stored and removed by protein in the body.

3.2. The determination of the force acting between a drug and BSA

The forces acting between a drug and biomolecule may include a hydrogen bond, van der Waals force, electrostatic force, hydrophobic interaction force, etc. If the temperature changes little, the reaction enthalpy change is regarded as a constant. From the equations:

$$\ln\frac{k_2}{k_1} = \left(\frac{1}{T_1} - \frac{1}{T_2}\right)\frac{\Delta H}{R} \quad (3)$$

$$\Delta G = \Delta H - T\,\Delta S = -RT\ln K \quad (4)$$

where the values of ΔH, ΔG and ΔS are enthalpy change, free energy change and entropy change, respectively, ΔH and ΔS are 21.6 and 73.55 KJ, respectively, for the binding reaction between terazosin and BSA. So it can be deduced that the acting force is mainly a hydrophobic interaction force [8].

3.3. Energy transfer between terazosin and BSA

According to Föster's non-radiative energy transfer theory [9,10], the energy transfer effect is related not only to the distance between the acceptor and donor (r_0), but also to the critical energy transfer distance (R_0), i.e.:

$$E = \frac{(R_0)^6}{(R_0)^6 + (r_0)^6} \quad (5)$$

where R_0 is the critical distance when the transfer efficiency is 50%.

$$(R_0)^6 = 8.8 \times 10^{-25} K^2 N^{-4} \Phi J \quad (6)$$

where K^2 is the spatial orientation factor of the dipole, N the refractive index of the medium, Φ the fluorescence quantum yield of the donor, J the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

$$J = \frac{\sum F(\lambda)\,\varepsilon(\lambda)\,\lambda^{-4}\,\Delta y}{F(y)}\,\Delta y \quad (7)$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at wavelength λ, $\varepsilon(\lambda)$ the molar absorptivity of the acceptor at wavelength λ, so the energy transfer efficiency is:

$$E = \frac{1 - F}{F_0} \quad (8)$$

The overlap of the absorption spectrum of terazosin and the fluorescence emission spectrum of BSA is shown in Fig. 3. J can be evaluated by integrating the spectra in Fig. 3 for $\lambda = 300$–400 nm and is 1.385×10^{-14} cm^{-3} l mol^{-1}. Under these experimental conditions, we found $R_0 = 1.83$ nm from Eq. (6) using $K^2 = 2/3$, $N = 1.336$, $\Phi = 0.15$ [11], the energy transfer effect $E = 0.015$ from Eq. (8) and the

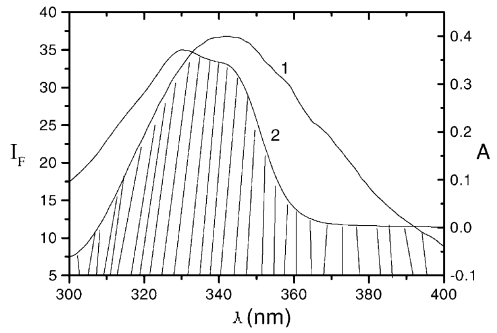

Fig. 3. Overlap of the fluorescence emission spectrum of BSA (1) with the absorption spectrum of terazosin (2).

distance between terazosin and amino acid residue in BSA, $r = 3.49$ nm.

3.4. The effect of other ions on the formation constant between drug and protein

The UV absorption spectrum of terazosin in the presence of metal ions shows there is no interaction between metal ions and terazosin. But there is a binding reaction between metal ions and protein and the presence of metal ions directly affects the binding between drug and protein. Further, in order to study the effect of metal ions on the binding between drug and BSA, some binding constants were determined at various temperature and in presence of various metal ions. The results are shown in Table 1.

The competition between metal ions and drug decreased the binding constant between protein and drug, implying that the binding force between protein and drug also decreased. Thus, shortening the storage time of the drug in blood plasma and enhancing the maximum effectiveness of the drug.

3.5. Effect of the presence of protein on the determination of terazosin

Terazosin has strong fluorescence at $\lambda_{ex/em} = 280/413$ nm. Fig. 1 shows that there is considerable protein fluorescence at 413 nm, when the protein concentration is 5.0×10^{-6} mol l^{-1}. In addition, the terazosin fluorescence spectra is somewhat quenched and the peak is blue-shifted. This further showed that a compound was formed between protein and terazosin. In the synchronous spectra [5], the sensitivity associated with fluorescence is maintained while offering several advantages: spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects. The blank intensity is about 13 at $\lambda_{em} = 413$ nm when obtained by normal fluorescence, while that obtained by synchronous fluorescence is nearly 0 at $\lambda_{em} = 413$ nm. For the selection of the optimum $\Delta\lambda$ value in the synchronous spectra, the following $\Delta\lambda$ values were studied: 5, 10, 20, 25, 30, 40, 60 nm. When $\Delta\lambda$ is <10 nm, the spectral shape is irregular and the fluorescence intensity is very weak. When $\Delta\lambda$ is >30 nm, two peaks cannot be separated completely. When $\Delta\lambda$ is between 10 and 30 nm, two distinct peaks appeared with a good shape. Therefore, maintaining $\Delta\lambda = 20$ nm, the range of synchronous scanning is λ_{ex}: 340–430 nm, λ_{em}: 360–450 nm (Fig. 4).

3.6. Determination of terazosin

3.6.1. Optimization of experimental conditions

The maximal fluorescence wavelengths of terazosin are $\lambda_{ex/em} = 280/413$ nm. Experimental results showed that the fluorescence intensity of terazosin was hardly effected by increasing the temperature from 25 to 45 °C and the ionic strength from 0 to 1.0 M. The intensity increased regularly with increase of pH from 7 to 14 because of the presence of the

Table 1
Binding constants in the presence of metal ions: K'_A (25 °C) and K''_A (35 °C)

M^{n+}	K'_A	r (nm)	K'_A/K	K''_A	r_0 (nm)	K''_A/K_A
Fe^{3+}	1.05×10^4	0.9952	0.493	0.87×10^4	0.9982	0.515
Al^{3+}	2.12×10^4	0.9981	0.995	1.67×10^4	0.9999	0.998
Zn^{2+}	1.16×10^4	0.9974	0.545	1.36×10^4	0.9994	0.805
Mg^{2+}	1.55×10^4	0.9997	0.728	1.58×10^4	0.9936	0.935
Cr^{3+}	0.89×10^4	0.9980	0.418	1.66×10^4	0.9928	0.982

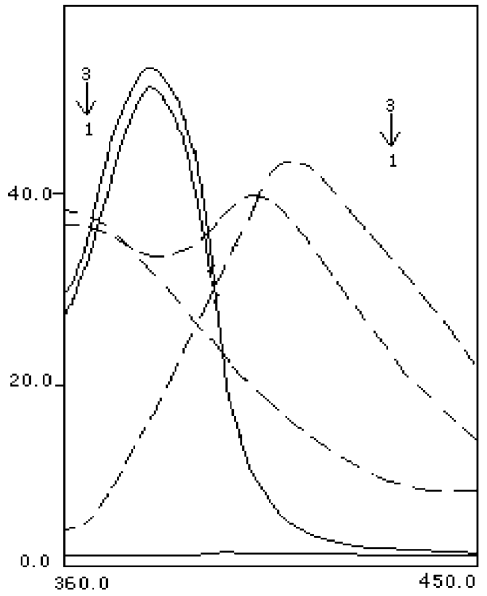

Fig. 4. General fluorescence spectrum (---) (λ_{ex} = 280 nm); synchronous fluorescence spectrum (—) ($\Delta\lambda$ = 20 nm); C (10^{-6} mol l^{-1}). (1) BSA = 5; (2) BSA = 5 + terazosin = 2; (3) terazosin = 2.

–NH_2 group. Thus, physiologic conditions were selected, i.e. ionic strength of 0.1 M, 35 °C and pH 7.4. The wavelength difference recommended is 20 nm. Under such conditions, the sensitivity is high and the effect of the protein is eliminated.

3.6.2. Precision, limit of detection and working curve

Under these recommended conditions, there is a linear relationship between fluorescence intensity and concentration of terazosin in the range 0–9 × 10^{-6} mol l^{-1} with a correlation coefficient (r) of 0.9989 (n = 6). The detection limit (DL) as defined by IUPAC [12], was determined to be 0.21 mg l^{-1} when the K value was taken as 3. The relative standard deviation was 2.5%, obtained from a series of 11 standards of 2.0 × 10^{-6} mol l^{-1} terazosin.

Table 2
Determination results of terazosin in samples (n = 3)

Sample	Initial (μg)	Added (μg)	Found (μg)	Recovery (%)
Serum	0	14.28	14.29	100.1
	0	18.36	18.56	101.1
	0	24.48	24.80	101.3
Urine	0	10.864	10.510	96.7
	0	13.968	13.484	96.5
	0	18.624	18.095	97.2

4. Analysis of samples

Serum and urine samples (each 0.2 ml) to which terazosin had been added were analyzed. The amount of terazosin in both of them could be determined by a standard addition method (Table 2).

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