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Drug-Drug Interactions Between Ciprofloxacin and Captopril at Binding Sites of Bovine Serum Albumin

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Abstract: The study of drug-drug interaction between Ciprofloxacin and Captopril at binding sites of Bovine Serum Albumin (BSA) was studied by equilibrium dialysis (ED) method. During concurrent administration of these drugs, Captopril (an ACE inhibitor) and Ciprofloxacin HCl (a broad spectrum antibacterial drug) have been found to increase the free concentration of Captopril due to Ciprofloxacin causing reduced binding to BSA. The increment in free concentration of Captopril was more prominent in presence of site-II specific probe (diazepam) than in absence of same probe. When no probe and no Ciprofloxacin was added the free concentration of Captopril was only 5% whereas this release was 23% when Ciprofloxacin was added with an increasing concentration from 1 $x10^{-5}$ to 12 $x10^{-5}$ M. The increment of free concentration of Captopril was from 9.5% to 43% in presence of site-II specific probe upon the addition of Ciprofloxacin with an increasing concentration from $0x10^{-5}$ to 12 $x10^{-5}$ M. During concurrent administration site-to-site displacement has taken place.

Key words: Ciprofloxacin, captopril, drug-drug interaction, BSA, equilibrium dialysis

INTRODUCTION

The primary structure of human serum albumin (HSA) was conducted by Meloun *et al*., 1975 and Brown, 1976. It is folded into three domains, each of which is built of three loops. HSA is comparatively a large multi-domain protein. Bovine serum albumin (BSA) and HSA has structural similarity (Brown, 1977). Among the plasma proteins, albumin is mostly bound to ligands or drugs.

The reduction in the extent of binding of a drug to protein occurred by the presence of other drugs is termed as drug-drug interaction or drug displacement. Competitive displacement and Non-Competitive displacement at binding sites may take place. As a consequence the free concentration of the displaced drug increases and may even lead to higher pharmacological as well as toxic effects (Rahman *et al*., 1993).

Protein of a drug is not a phenomenon particular to the plasma. Plasma protein binding properties are related to plasma clearance, elimination half-life, apparent volume of the distribution and area under the curve. Though the information resource regarding the binding of drugs to HSA is extensive, the mechanism of drug binding to HSA is still a subject of speculation and controversy (Jiunn *et al*., 1987).

Combination therapy is common practice now a day. During the concurrent use of drugs all the drugs may exert their effects independently or may interfere or interact with each other in biopharmaceutical, biochemical or in pharmacological point of view.

Keeping this consideration in mind broad spectrum antibacterial drug, ciprofloxacin and antihypertensive drug, a angiotensin convertin genzyme (ACE) inhibitor, Captopril have been used in the study to observe the site to site displacement of these two drugs.

MATERIALS AND METHODS

Drug-drug displacement study

Effect of ciprofloxacin on captopril binding to BSA in absence of site-II specific probe (diazepam), was studied as follows: Three ml of previously 2x10-5 M BSA solution was taken in each of nine cleaned and dried test tubes. Captopril solution was taken in each of eight cleaned and dried test tubes so that the final ratio between protein and Captopril was 1: 1($2x10^{-5}$ M: $2x10^{-5}$ M). The ninth test tube containing only BSA solution was marked as 'blank'. Then Ciprofloxacin was added with an increasing concentration into seven test tubes to make the final ratio of protein, Captopril and Ciprofloxacin 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8,1:1:10 and 1:1:12. Ciprofloxacin was not added in the first test tube.

Mixing for 15 minutes the solution was pipette out and poured into eight different semi-permeable membrane tubes. The tubes containing drug mixture were immersed in eight 50 mL conical flask containing 30 mL of phosphate buffer solution of pH 7.4 and shake in a metabolic shaker at 25°C and 125 rmp for about 10 hours uninterruptedly.

Collecting the buffer solution from the conical flask free concentrations of Captopril were measured by a UV spectrophotometer at a wave- length of 220 nm.

Effect of ciprofloxacin on captopril binding to BSA in presence of site-II probe: Three ml of previously 2 x10^{-5} M BSA solution was taken in each of nine cleaned and dried test tubes. Diazepam solution was taken in each test tube, so that the final ratio between protein and diazepam was 1:2 (2x10^{-5} M: 4x10^{-5} M). Captopril solution was taken to make the final ratio of protein diazepam and Captopril, 1: 2: 1(2x10^{-5} M: 4x10^{-5} M: 2x10^{-5} M) in each of the eight test tubes. The ninth test tube containing only BSA solution was marked as 'blank'. Ciprofloxacin was added with an increasing concentration into seven out of eight test tubes containing 1:2:1 mixture of protein, diazepam and Captopril to make the final ratio of protein, diazepam, Captopril and Ciprofloxacin, 1:2:1:0, 1:2:1:1, 1:2:1:2, 1:2:1:4, 1:2:1:6, 1:2:1:8, 1:2:1:10 and 1:2:1:12. Ciprofloxacin was not added in first test tube.

Then as the same way as that of in absence of probe, shaking and collecting the buffer solution free concentrations of Captopril were measured by a UV spectrophotometer at a wave- length of 220 nm.

Drugs and probe used in the experiment: Diazepam (Square Pharmaceuticals Limited), Captopril (IBN Sina Pharmaceuticals Ltd.) and Ciprofloxacin (Beximco Pharmaceuticals Ltd.).

Reagents used: Disodium hydrogen phosphate (Na_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Borax ($NaB_4O_7.H_2O$) (Analytical grade, Glaxo), Cellulose Membrane (Medical International Limited. Liverpool Road, London; mol wt 1200 Daltons), Bovine Serum Albumin (BSA) (fatty acid free, fraction V, 96-98%, Mol. Wt 66500 and purchased from the Sigma Chemical Co).

Instruments used: pH Meter (HANNA Microprocessor pH Meter, Portugal), SP8-400 UV/VIS Spectrophotometer (Thermospectronic, England.), Metabolic Shaking Incubator (Clifton Shaking Bath, Nickel Electro Ltd., England.), Micro syringe (Well. Liang.Jin.Yang.q.I., Chaina.).

Equilibrium Dialysis method was employed in the study (Singlas, 1987a, b).

RESULTS AND DISCUSSION

Adequate knowledge about composition, size and location of binding sites as well as the probable interactions at binding sites at HSA along with all the binding parameters of plasma protein is required for proper explanation of pharmacokinetic aspects of drugs. It is important, for the rational understanding of drug-serum albumin binding during concurrent administration and its consequences in drug actions.

Drug-drug interaction: Plasma protein binding properties are considered as the primary determinants of the pharmacokinetic properties of drugs. Any physiological or any other condition that causes alteration in the serum albumin binding of the drugs might lead to change in the pharmacokinetic and pharmacological properties of the drugs. Drug-drug interactions thus play a vital role in the extent of plasma-protein binding and consequently the therapeutic and/or toxic effects of the drugs. The probable interactions between drugs at the binding sites of the drug on the plasma protein should to be known (Sjoholm, 1980).

Ciprofloxacin-Captopril interaction: In the study of Ciprofloxacin-Captopril interaction the effect of Ciprofloxacin on Captopril bound to BSA was determined in absence and in presence of site-II probe (diazepam) at 25°C and pH 7.4. Site-II specific probe, diazepam was used when probe was used. Free concentration of Captopril bound to BSA (1:1; 2 x10^{-5}M: 2 x10^{-5}M) released upon the addition of Ciprofloxacin in absence of site-II specific probe from 5 to 23%. But on the other hand in presence of site-II specific probe (diazepam) free concentration of Captopril bound to BSA increased from 9.5 to 43% with the increase of Ciprofloxacin concentration from 0 x10^{-5}M to 12 x10^{-5}M (Fig. 1).

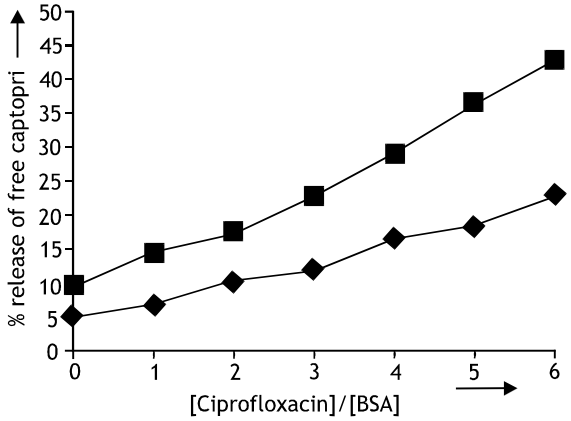

Fig. 1: Effect of Ciprofloxacin on Captopril binding to BSA in absence (◆) and in presence (■) of site-II specific probe, diazepam upon the addition of increasing concentration of Ciprofloxacin from 0x10^{-5} to 12 x10^{-5} M

During concurrent administration of Ciprofloxacin and Captopril in absence of site-II specific probe diazepam, Ciprofloxacin displaced Captopril from site-II and thus free concentration of Captopril increased from 5 to 23% (Fig. 2).

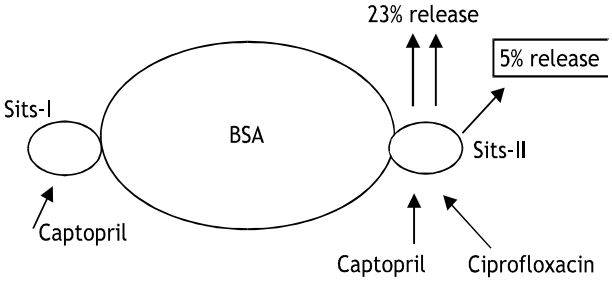

Fig. 2: Proposed models of the Ciprofloxacin-BSA-Captopril interaction in absence of site-II specific probe diazepam

[BSA] = [Captopril] = $2x10^{-5}$M and [Ciprofloxacin] = 0-$12x10^{-5}$M

In Fig. 3 it is indicated that the effect of Ciprofloxacin on Captopril bound to BSA in presence of site-specific probe-II. When site-II blocking probe diazepam was used the presence of diazepam caused the free concentration Captopril bound to BSA release from 9.5 to 43%.

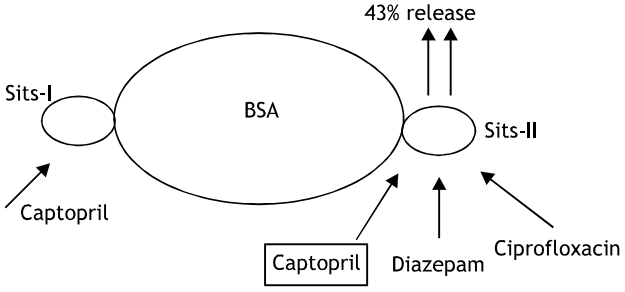

Fig. 3: Proposed models of the Ciprofloxacin-BSA-Captopril interaction in presence of site-II specific probe diazepam

[BSA] = [Captopril] = $2x10^{-5}$M, [Diazepam] = $4x10^{-5}$M and [Ciprofloxacin] = 0-$12x10^{-5}$ M

This little more increment of release of free Captopril than that of absence of probe can be justified and it might be due to the inhibition of released drug from further binding to site-II, which is blocked by diazepam or it might be due to the displacement by the probe, diazepam or might be due to the displacement by Ciprofloxacin for co-operative binding between Ciprofloxacin and the probe.

Pharmacokinetic implications: During concurrent administration of these two drugs site-to-site displacements will take place and may change pharmacodynamic properties of the drugs.

Care should be exercised during concurrent administration of Ciprofloxacin and Captopril. Higher research may reveal other important aspects of the present study.

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