Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2016, 6(4), 589-595
doi: 10.15171/apb.2016.073
http://apb.tbzmed.ac.ir

TUOMS Publishing Group

Research Article

Characterization of the Effect of Drug-Drug Interaction on Protein Binding in Concurrent Administration of Sulfamethoxazol and Diclofenac Sodium Using Bovine Serum Albumin

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Article info

Article History:
Received: 22 May 2015
Revised: 18 September 2016
Accepted: 7 November 2016
ePublished: 22 December 2016

Keywords:
· Bovine serum albumin
· Diazepam
· Diclofenac sodium
· Equilibrium dialysis
· Sulfamethoxazole
· Warfarin sodium

Abstract

Purpose: This project was aimed to determine the effect of concurrent administration of sulfamethoxazole and diclofenac sodium.

Methods: Equilibrium dialysis method was adopted to study different protein binding aspects of sulfamethoxazole and diclofenac sodium.

Results: Sulfamethoxazole showed two types of association constants; high affinity constant $29.0\pm0.20\times10^6$ M^{-1} with lower number of binding sites of 0.7±1 and low affinity constant $1.13\pm0.20\times10^6$ M^{-1} with higher number of binding sites of 3.45±1 at pH 7.4 and 40 °C temperature. Diclofenac sodium showed high affinity constant $33.66\pm0.20\times10^6$ M^{-1} with lower number of binding sites of 1.01±1 and low affinity constant $1.72\pm0.20\times10^6$ M^{-1} with higher number of binding sites of 6.40±1 at the same condition. Site specific probe displacement data implied that site-I, warfarin sodium site, was the high affinity site, while site-II, diazepam site, was the low affinity site for these drugs. During concurrent administration, sulfamethoxazole increased the free concentration of diclofenac sodium from 17.5±0.14% to 70.0±0.014% in absence and from 22.5±0.07% to 83.0±0.014% in presence of site-I specific probe. Diclofenac sodium also increased the free concentration of sulfamethoxazole from 2.8±0.07% to 52.0±0.14% and from 8.5±0.014% to 64.4±0.07% in absence and presence of site-I specific probe respectively.

Conclusions: The study revealed that the concurrent administration of sulfamethoxazole and diclofenac sodium may result drug concentration alteration in blood.

Introduction

Drug-protein binding means the formation of plasma protein complex with drug after reaching the blood. It is one of the pharmacokinetic parameters of a drug. The amino acids that compose the protein chain have hydroxyl, carboxyl or other sites available for reversible drug interactions. Drug may bind to albumin, alpha acid glycoprotein, lipoproteins and immunoglobulins.[1] According to probe displacement method there are at least three relatively high specific drug-binding sites on the human serum albumin (HSA) molecule. These sites are generally called the warfarin binding site, the benzodiazepine-binding site and the digoxin binding site which are also denoted as site-I, site-II and site-III, respectively. Site-II is more independent binding site and more specific than Site-I and Site-III.[2,3] As free fraction of drug available in plasma is responsible for the pharmacological response, the drug displacement may cause excessive toxicity of the displaced drug due to increase in free drug concentration in blood. Drug-protein deals with the pharmacokinetic and pharmacodynamic behavior of a drug. Understanding the location of drug binding sites on HSA as well as their extent and composition is essential for proper realization and prediction of drug-drug interaction, explanation of any change in HSA binding of drug under various disease conditions and clarification of various pharmacokinetic parameters.[4] There are two main types of protein binding; strong affinity binding to a small number of sites and weak affinity binding to a large number of sites. Since binding is almost exclusively to albumin and the number of sites available is limited, the protein binding of some drugs depends on the plasma albumin concentration. Plasma protein binding properties are primary determinants of the pharmacokinetic properties of most of the drug such

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as plasma clearance, elimination half-life, apparent volume of the distribution, and area under the curve.[5]

In this study, the binding sites, association constants, number of binding sites of sulfamethoxazole and diclofenac sodium and their interaction with each other both in presence and absence of site specific probe were determined in concurrent administration. Because, the information resource regarding the binding of drugs (sulfamethoxazole and diclofenac sodium) to HSA is extensive, but the mechanism of drug binding to HSA is still a subject of speculation and controversy.

This study is performed to estimate the effect of sulfamethoxazole, an effective antibacterial agent and competitive antagonist of para-aminobenzoic acid (PABA) on diclofenac sodium,[6] a non-steroidal anti-inflammatory drugs (NSAIDs), commonly used for the therapy of chronic forms of arthritis and mild-to-moderate acute pain, fever and inflammation and can be used without prescription.[7] Sulfamethoxazole combined with trimethoprim as cotrimoxazole is used in case of infection and infection often causes pain. So, concurrent or successive administration of diclofenac Na and sulfamethoxazole may be required. For instance, cotrimoxazole and diclofenac co-administration was reported to produce complete stone expulsion rate in patients with urethral colic due to juxtavesical stones.[8] Successive administration of diclofenac sodium and sulfamethoxazole was reported to be significantly beneficial in Whipple's disease with normal duodenal histology and ankylosing spondylitis.[9] Moreover, co-administration of warfarin with sulfamethoxazole, investigated in this study is reported to have clinically significant drug interactions.[10] So, understanding the drug interaction between these two drugs is important which has not yet done. The aim of this project was to understand protein binding parameters of concurrently used sulfamethoxazole and diclofenac sodium on bovine serum albumin (BSA) adopting equilibrium dialysis.[11,12]

Materials and Methods

Equilibrium dialysis method was employed in this study according to the method of Uddin et al.[12] and Singlas.[13]

Drugs and chemicals used

Sulfamethoxazole, diclofenac sodium, warfarin sodium (site-I probe) and diazepam (site-II probe) were purchased from different Bangladeshi pharmaceutical companies namely Beximco pharmaceuticals Ltd., ACI pharmaceuticals Ltd., Incepta pharmaceuticals Ltd. and Navana pharmaceuticals Ltd. respectively. Buffering agent disodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were manufactured by Glaxo; U.K. Dialysis tubing cellulose membrane used in the experiment was purchased from Medicell international Ltd., U.K. which has a molecular weight cut off at 1200 daltons. Bovine serum albumin (fatty acid free, fraction V, 96-98%) purchased from the Sigma chemical Co. USA was used as a protein. The molecular weight of the protein was assumed to be 66500. De-ionized water, distilled water, methanol supplied by Laboratory Patterson Scientific, U.K. were used as solvent and chemicals.

Instruments and equipment

Double beam Analykjena UV-visible spectrophotometer (Model 205, Germany) was used to measure the absorbance of drug solution. pH meter (Serial no.- 453088, Hanna, Portugal), electronic balance (Serial no.- 1508, OHAUS, Germany), metabolic shaking incubator (Serial no.- 490639525, New Brunswick Scientific, USA), micro syringe (Serial no.- HN42250, Jencons, UK), hot plate (Serial no.- SWT.550010W, Gallenkamp, England) were used in this study.

Preparation of membrane

Cellulose membrane was cut into small pieces of- 2 inches length and taken in 1000 ml beaker containing de-ionized water. The pieces of membrane were immersed beneath the de-ionized water and heated for more than 8 hours in order to remove sulfur. The temperature was maintained between 65-70 °C and hot water was replaced by fresh de-ionized water at every 1 hour interval.

Preparation of buffer, protein (BSA) and stock (sulfamethoxazole and diclofenac sodium, warfarin sodium, diazepam) solutions

Phosphate buffer of pH 7.4 was prepared by the method as described by Perrin and Dempsey.[14] A protein solution of 2×10^{-5} M BSA at pH 7.4 was prepared carefully and gently to avoid foam formation. The protein solution was kept in a refrigerator until use. Stock solutions of 1×10^{-3} M of sulfamethoxazole, diclofenac sodium, warfarin sodium and diazepam were prepared.

Preparation of standard curve of sulfamethoxazole, diclofenac sodium, warfarin sodium and diazepam

The concentrations of 0.5×10^{-6}, 1×10^{-6}, 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, 8×10^{-6}, 10×10^{-6}, 12×10^{-6} and 14×10^{-6} M for sulfamethoxazole, diclofenac sodium, warfarin sodium and diazepam were prepared first at pH 7.4. The solutions were then properly mixed. The absorbance of the solutions were determined according to British Pharmacopoeia at λ_{max} 265, 254, 308 and 235 nm for sulfamethoxazole, diclofenac sodium, warfarin sodium, and diazepam respectively by a UV spectrophotometer. As a control or reference sample, phosphate buffer solution of only BSA at pH 7.4 was used. The standard curve was obtained by plotting the absorbance against the corresponding concentrations.

Estimation of association constant of sulfamethoxazole and diclofenac sodium

Association constant and corresponding binding site number of sulfamethoxazole and diclofenac sodium were estimated using Scatchard's method of analysis. To determine the association constant of sulfamethoxazole and diclofenac sodium at pH 7.4, 8 clean and dried test tubes were taken and 3 ml of previously prepared 2×10^{-5} M BSA solution was taken in each of them. Drug stock solutions (1×10^{-2} and 1×10^{-3} M) was added in different volumes to 7 out of 8 test tubes to have the following concentrations: 0.5×10^{-6}, 1×10^{-6}, 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, 8×10^{-6} and 10×10^{-6} M. The eighth test tube containing only BSA solution was marked as "control". After mixing the solutions they were allowed to stand for 10 minutes for maximum binding of drug to BSA.[15,16]

2 ml from each test tube was pipetted out and poured into previously prepared semi-permeable membrane tubes and finally both sides of the tubes were clipped properly to prevent leakage. The membrane tubes containing the drug-protein mixture were immersed in 50 ml flasks containing 30 ml of phosphate buffer solution of pH 7.4. The conical flasks were sealed by foil paper and placed in a metabolic shaker for dialysis for 10 hours at 40 °C and 20 rpm. Buffer samples were collected from each flask after complete dialysis. Absorbance of free concentrations of sulfamethoxazole and diclofenac sodium were measured by a UV-visible spectrometer.

Determination of binding site of sulfamethoxazole and diclofenac sodium using warfarin sodium as a site-I specific probe and diazepam as a site-II specific probe

To determine the binding site of sulfamethoxazole and diclofenac sodium using warfarin sodium and diazepam, 3 ml of previously prepared 2×10^{-5} M BSA solution was taken to each of the 8 clean and dried test tubes.[15,17] 1×10^{-3} M warfarin sodium and diazepam solution were added to the 7 out of 8 test tubes so that the final ratio of protein and probe (warfarin sodium and diazepam) was 1:1 (2×10^{-5} M:2×10^{-5} M) in each of these 7 test tubes. The eighth test tube containing only BSA solution was marked as "blank" or "control". These mixtures were allowed to stand for 10 minutes to allow binding of the warfarin sodium and diazepam to their particular binding sites.

Sulfamethoxazole and diclofenac sodium solutions (either 2×10^{-2} M or 2×10^{-3} M) were added with increasing concentrations into 6 out of 7 test tubes containing 1:1 mixture of protein-warfarin sodium. The final ratios of protein:probe (warfarin sodium or diazepam):drug (sulfamethoxazole or diclofenac sodium) were 1:1:0, 1:1:2, 1:1:4, 1:1:6, 1:1:8, 1:1:10 and 1:1:12.

After pipetting, the solution was properly mixed and allowed to stand for 10 minutes to ensure maximum binding of drug to sites and thereby displacing the probe from sites on BSA. From each test tube 2 ml of the solution was taken into seven different semi-permeable membrane tubes. Two ends of the membrane a tube were clipped ensuring no leakage. The membrane tubes were then immersed in seven separates 50 ml conical flasks containing 30 ml of phosphate buffer solution of pH 7.4.

The conical flasks were then placed in a metabolic shaker for dialysis at 40 °C and 20 rpm and shaking was continued for 10 hours. At the end of dialysis, samples were collected from each flask. The free concentrations of warfarin sodium and diazepam were measured by a UV spectrophotometer at λ_{max} 308 and 235 nm.

Drug- drug displacement study

Effect of sulfamethoxazole on diclofenac sodium binding to BSA was analyzed both in absence and presence of warfarin sodium (site-I specific probe). When the experiment was carried out in absence of warfarin sodium, 3 ml of 2×10^{-5} M BSA solution was taken in each of 8 clean and dried test tubes. 6 µl of 1×10^{-2} M diclofenac sodium solution was taken to each of 7 test tubes so that the final ratio between protein and diclofenac sodium was 1:1 (2×10^{-5}M: 2×10^{-5}M) in each of the 7 test tubes. The eighth test tube containing only BSA solution was marked as blank.[15,16] Sulfamethoxazole was added with an increasing concentration into 6 out of 7 test tubes containing 1:1 mixture of protein-drug to make the final ratio of protein, diclofenac sodium and sulfamethoxazole 1:1:0, 1:1:1, 1:1:2, 1:1:4, 1:1:6, 1:1:8 and 1:1:10.

In presence of warfarin sodium, 3 ml of 2×10^{-5} M BSA solution was taken to each of 8 clean and dried test tubes. 12 µl of 1×10^{-2} M warfarin sodium solution was taken to each test tube, so that the final ratio between protein and warfarin sodium was 1:2 (2×10^{-5} M:4×10^{-5} M). 6 µl of 1×10^{-2} M diclofenac sodium solution was taken to each of 7 clean and dried test tubes and the final ratio among protein, warfarin sodium and diclofenac sodium was 1:2:1 (2×10^{-5} M:4×10^{-5} M:2×10^{-5} M) in each of the 7 test tubes. The eighth test tube containing only BSA and warfarin sodium solution was marked as "blank". Sulfamethoxazole was added with an increasing concentration into 6 out of 7 test tubes to make the final ratio 1:2:1:0, 1:2:1:1, 1:2:1:2, 1:2:1:4, 1:2:1:6, 1:2:1:8 and 1:2:1:10 of protein, warfarin sodium, diclofenac sodium and sulfamethoxazole.

In the both tests, all the solution mixtures were then properly mixed and allowed to stand for 15 minutes for the confirmation of maximum binding to BSA. After that the solution was pipetted out and poured into 7 different semi-permeable membrane tube and both ends of the membrane tubes were clipped to ensure no leakage. The tubes containing drug-protein mixture were immersed in 50 ml conical flask containing 30 ml of phosphate buffer solution of pH 7.4. The conical flasks were placed in a metabolic shaker at 40 °C and 20 rpm for about 10 hours uninterruptedly. After

shaking samples in the buffer solution were collected. Free concentrations of diclofenac sodium were measured by a UV-visible spectrometer.

Effect of diclofenac sodium on sulfamethoxazole binding to BSA was also measured in absence and presence of site-I specific probe warfarin sodium using the same protocol. Free concentrations of sulfamethoxazole were measured by a UV-visible spectrometer.

Data Analysis

The fraction of unbound drug was calculated from the absorbance of UV spectrometer. Both association constant (k) and number of binding sites (n) of sulfamethoxazole and diclofenac sodium were determined by using scatchard plot. Scatchard plot is constructed by plotting the ratio between the molar concentration of bound drug and number of protein molecule (r) in abscissa and r/D_f (D_f represents the free drug concentration). The r and Df data were fitted by linear least squares regression analysis when number of binding sites and association constants were calculated.[11,12,15,17]

Results and Discussion

Adequate knowledge about composition, size and location of binding sites are required for a proper explanation of serum protein binding. It is important, as it is an essential tool for the rational understanding and serum albumin binding during various physiological conditions and concurrent administration. So, the mechanism of drug-albumin binding is of vital importance.[2-5,18-21]

Estimation of binding parameters

To estimate the binding parameters of sulfamethoxazole, equilibrium dialysis (ED) method was used and the subsequent non-linear shape of the Scatchard plot describes both high and low affinity binding site of the drugs on the protein molecule.[11,12,15]

The results obtained by this analysis suggest that both sulfamethoxazole and diclofenac sodium have two types of association constants namely high affinity association constant (k_1) with low capacity (n_1) and low affinity association constant (k_2) with high capacity (n_2). Sulfamethoxazole is characterized by a k_1 to BSA and the value at pH 7.4 is $29\pm0.20\times10^6$ M^{-1} while k_2 is $1.13\pm0.20\times10^6$ M^{-1} which is nearly 26 times lower than that of k_1. So, for sulfamethoxazole, the lower number (n_1) and higher number (n_2) of binding sites are found 0.7±1 and 3.45±1 respectively (Figure 1).

On the other hand, diclofenac sodium is characterized by a (k_1 to BSA and the value at pH 7.4 is $33.66\pm0.20\times10^6$ M^{-1}, while k_2 is $1.72\pm0.20\times10^6$ M^{-1} for diclofenac sodium is nearly 19.5 times lower than that of the higher affinity association constant. So, for diclofenac sodium, the lower numbers (n_1) and higher numbers (n_2) of binding sites are 1.01±1 and 6.40±1 respectively (Figure 2).

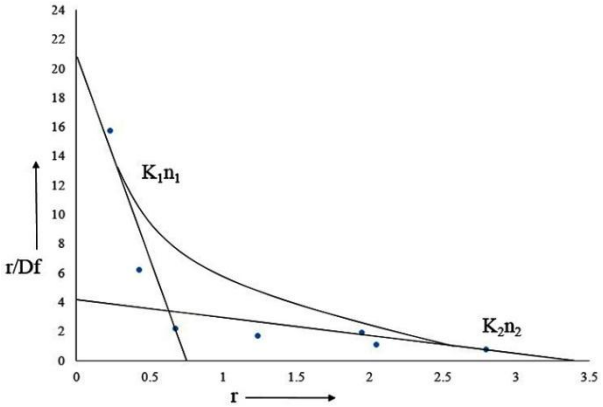

Figure 1. Scatchard plot of sulfamethoxazole at pH 7.4 and 40°C D_f = Free drug concentration, r = Ratio of molar concentration of bound drug and protein, K_1= High affinity association constant, n_1= Number of low capacity binding site per protein, K_2= Low affinity association constant, n_2= Number of high capacity binding site per protein.

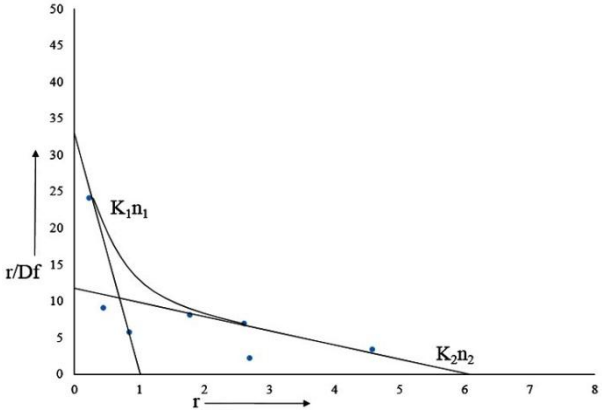

Figure 2. Scatchard plot of diclofenac sodium at pH 7.4 and 40 °C D_f is the free drug concentration and r is the ratio of molar concentration of bound drug and protein, K_1= High affinity association constant, n_1= Number of low capacity binding site per protein, K_2= Low affinity association constant, n_2= Number of high capacity binding site per protein.

Identification and characterization of binding site

Well-established probes, which are specific for particular sites on the albumin molecule, are used for identification of binding site of the drugs on the protein molecule. If a drug is able to displace a probe from its binding site, it is assumed that, the drug also binds to that particular site. Thus, the binding site as well as the specificity and relative strength of binding to albumin of sulfamethoxazole have been determined by this principle. Here, as site-I specific probe, warfarin sodium and site-II specific probe, diazepam were used.[11,12,15]

To characterize the binding site of sulfamethoxazole, the free concentration of warfarin sodium (site-I specific probe) bound to BSA was measured upon the addition of sulfamethoxazole. It was found that, the free concentration of warfarin sodium was increased from 100% (as % of initial) to 238.5% when the ratio of sulfamethoxazole to BSA was increased from 0 to 6 (Figure 3).

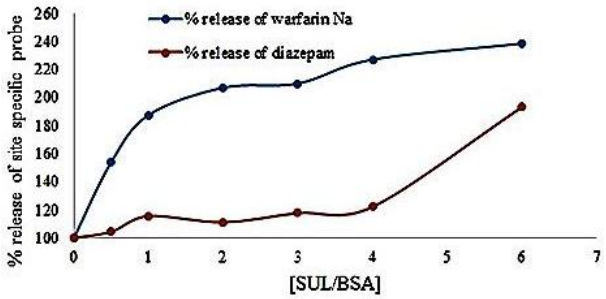

Figure 3. Data for site determination of sulfamethoxazole on BSA using warfarin sodium and diazepam
SUL= Sulfamethoxazole, BSA= Bovine serum albumin.

In contrast, under the same experimental conditions, when in lieu of warfarin sodium, diazepam was used as site-II specific probe, the increment of the free concentration of diazepam by sulfamethoxazole was increased from 100% (as % of initial) to 193.3% when the ratio of sulfamethoxazole to BSA was increased from 0 to 6 (Figure 3).

On the other hand to characterize the binding site of diclofenac sodium, the free concentration of warfarin sodium (site-I specific probe) bound to BSA was measured upon the addition of diclofenac sodium. It was found that, the free concentration of warfarin sodium was increased from 100% (as % of initial) to 190.3% when the ratio of diclofenac sodium to BSA was increased from 0 to 6 (Figure 4).

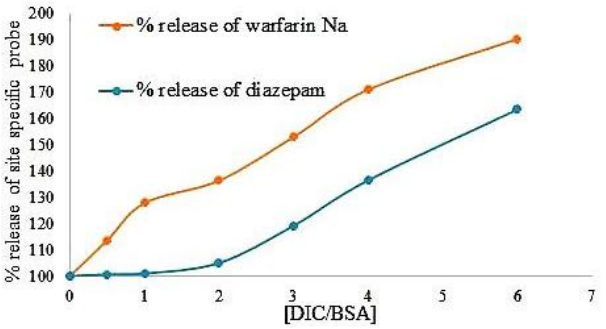

Figure 4. Data for site determination of diclofenac sodium on BSA using warfarin sodium & diazepam
DIC= Diclofenac sodium, BSA= Bovine serum albumin.

In contrast, under the same experimental conditions, when in lieu of warfarin sodium, diazepam was used as site-II specific probe, the increment of the free concentration of diazepam by diclofenac sodium was from 100% (as % of initial) to 163.5% when the ratio of diclofenac sodium to BSA was increased from 0 to 6 (Figure 4).

From the study, it was found that, sulfamethoxazole increased the free concentration of warfarin sodium from 100% to 238.5%, whereas the free concentration of diazepam from 100% to 193.3%. On the other hand, diclofenac sodium also increased the free concentration of warfarin sodium from 100% to 190.3%, while under the same experimental conditions, diclofenac sodium increased the free concentration of diazepam from 100% to 163.5%. From these data, this is evident that, the increment of free concentration of warfarin sodium is greater than that of diazepam by both sulfamethoxazole and diclofenac sodium. So it can be concluded that both drugs preferentially bind to site-I. Again, as the displacements of diazepam are quite enough, it can be also suggested that, sulfamethoxazole and diclofenac sodium in addition to the site-I also bind to site-II on the BSA molecule but to a lower extent.

Drug-drug displacement study

Both sulfamethoxazole and diclofenac sodium were found site-I specific drug, so warfarin sodium was chosen as the probe when only one probe was used at physiological pH. In sulfamethoxazole-diclofenac sodium interaction study, the effect of sulfamethoxazole on diclofenac sodium bound to BSA and vice versa was determined in absence and presence of site-I specific probe warfarin sodium (Table 1 and 2). Diclofenac sodium bound to BSA (1:1; 2×10^{-5} M:2×10^{-5} M) released from 17.5±0.14% to 70.0±0.014% upon the addition of sulfamethoxazole in absence of site-I specific probe (warfarin sodium) . Again, in presence of site-I specific probe free concentration of diclofenac sodium increased from 22.5±0.07% to 83.0±0.014% with the increase of sulfamethoxazole concentration from 0×10^{-5} M to 10×10^{-5} M.

Table 1. Effect of sulfamethoxazole on diclofenac sodium bound to BSA

Ob. No.	Added SUL conc.× 10^{-5} M	[SUL] / [BSA]	In absence of site-I specific probe warfarin Na		In presence of site-I specific probe warfarin Na	
			Free conc. of DIC×10^{-5}M	% Displacement of DIC	Free conc. of DIC×10^{-5}M	% Displacement of DIC
1	0	0	0.35±.07	17.5±0.14	0.45±0.007	22.5±0.07
2	1.0	0.5	0.48±0.014	24.0±0.14	0.63±0.001	31.5±0.014
3	2.0	1	0.60±0.0014	30.0±0.07	0.75±0.001	37.5±0.014
4	4.0	2	0.76±0.003	38.0±0.014	0.97±0.003	48.5±0.07
5	6.0	3	0.93±0.007	46.5±0.014	1.18±0.007	59.0±0.14
6	8.0	4	1.12±0.001	56.0±0.014	1.38±0.001	69.0±0.028
7	10.0	5	1.40±0.001	70.0±0.014	1.66±0.007	83.0±0.014

Data are represented as mean±SD of replicates, Ob.= Observation, conc.= Concentration, SUL= Sulfamethoxazole, BSA= bovine serum albumin, DIC=Diclofenac sodium.

Table 2. Effect of diclofenac sodium on sulfamethoxazole bound to BSA

Ob. No.	Added DIC conc.×10^{-5} M	[DIC] / [BSA]	In absence of site-I specific probe warfarin Na		In presence of site-I specific probe Warfarin Na	
			Free conc. of SUL×10^{-5} M	% Displacement of SUL	Free conc. of SUL×10^{-5} M	% Displacement of SUL
1	0	0	0.056±0.001	2.8±0.07	0.170±0.007	8.5±0.14
2	1.0	0.5	0.16±0.003	7.3±0.14	0.334±0.014	16.7±0.14
3	4.0	2	0.392±0	19.6±0.14	0.620±0.007	31.0±0.07
4	6.0	3	0.560±0.007	28.0±0.28	0.800±0.014	40.0±0.14
5	8.0	4	0.820±0.007	41.0±0.07	1.090±0.014	54.4±0.28
6	10.0	5	1.040±0.007	52.0±0.14	1.288±0.007	64.4±0.07

Data are represented as mean±SD of replicates, Ob.= Observation, conc.= Concentration, DIC= Diclofenac sodium, BSA= Bovine serum albumin, SUL= Sulfamethoxazole.

Sulfamethoxazole bound to BSA (1:1; 2×10^{-5} M:2×10^{-5} M) released upon the addition of diclofenac sodium in absence of site-I specific probe (warfarin sodium) increased from 2.8±0.07% to 52.0±0.14%, while in presence of site-I specific probe, free concentration of sulfamethoxazole increased from 8.5±0.14% to 64.4±0.07% with the increase of diclofenac sodium concentration from 0×10^{-5} M to 10×10^{-5} M.

This study suggests that both sulfamethoxazole and diclofenac sodium increased the release of free drug from their binding protein both in presence and absence of site specific probe. From the binding characteristics of SUL and DIC and their interaction pattern recommend that DIC has less affinity to BSA protein than SUL which can be easily displaced by SUL and other site specific probes.

Drug-drug interaction

Plasma protein binding properties are considered as the primary determinants of the pharmacokinetic properties of drugs. Any physiological condition causing alteration in the protein binding of the drugs might lead to changes 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 effect of the drugs. To evaluate the probable interaction between drugs, the binding sites of the drug on the protein have to be known.[22]

Sulfamethoxazole is an effective antibacterial agent is used in different bacterial infections as well as is the drug of choice for treatment of many disease conditions like pneumocystosis and an acceptable oral therapy for recurrent urinary tract infections caused by susceptible bacteria.[23] As infectious diseases are associated with pain and treatment of pain and infection should be of equal priorities, the patients suffering from bacterial infection may require administering diclofenac during the regular use of sulfamethoxazole therapy.[24] In that case, if there is no alternative to sulfamethoxazole or diclofenac sodium and the drugs cannot be avoided altogether, the administration of these two drugs to be staggered over a period of time.

As, sulfamethoxazole has more affinity to plasma protein in comparison to diclofenac sodium and they have same binding site; so, sulfamethoxazole may displace diclofenac sodium slowly from its binding site resulting the increment of free diclofenac sodium concentration in blood. Dose adjustment of both drugs may be required in case of concurrent administration of sulfamethoxazole and diclofenac sodium to avoid drug toxicity.[25-27]

Conclusion

From the obtained data, it is too early to draw a concrete conclusion about the pharmacokinetic or pharmacological properties of the drug. Some recent high sensitive technologies like HPLC/UV analysis method can be adopted for quantitation and determination of the binding pattern of drugs to HSA.[28] A more detailed study including *in vivo* experiments is warranted in this respect. However, the results of the present study in combination with the current advances in the binding of sulfamethoxazole and diclofenac sodium, might be helpful in realizing the overall binding behavior of these two drugs with HSA.

Acknowledgments

This work was financially supported by the Khulna University Research Cell. The authors are grateful to the Laboratory of Pharmaceutical Technology, Pharmacy Discipline, Khulna University for giving technical support.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare no conflict of interests.

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