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COMPETITIVE BINDING OF IBUPROFEN AND NAPROXEN TO BOVINE SERUM ALBUMIN: MODIFIED FORM OF DRUG–DRUG DISPLACEMENT INTERACTION AT THE BINDING SITE

M. M. RAHMAN, M. H. RAHMAN*[+] AND N.N. RAHMAN*
Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
**Department of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*

The competitive binding characteristics of ibuprofen and naproxen with respect to binding site on bovine serum albumin (BSA) was studied by equilibrium dialysis method at pH 7.4 and 25°C. We studied the effect of one drug on the free concentration of another *in vitro* during concurrent administration. There was remarkable increase in free concentration of naproxen when ibuprofen ($2x10^{-5}$ M) was added to a 1:1 naproxen-BSA mixture ($2x10^{-5}$ M: $2x10^{-5}$ M), suggesting that ibuprofen displaced naproxen from its binding sites. However free fraction of naproxen was not increased up to the level expected from direct competitive displacement. Free concentration of ibuprofen was hardly increased by naproxen when naproxen ($2x10^{-5}$ M) was added to the ibuprofen-BSA (1:1) mixture. But in both cases, in presence of ranitidine (site I specific probe), the free concentration of the displaced drug increased more prominently compared to that in absence of ranitidine. This result suggests that, ibuprofen displaces naproxen and *vice versa* from its high affinity binding site (site II) and the displaced drug rebounds to its low affinity binding site (site I) on BSA molecule. This form of modified displacement has been arbitrarily referred to as site-to-site displacement.

Keywords: *Equilibrium dialysis, bovine serum albumin, ibuprofen, and naproxen.*

INTRODUCTION

The association of drugs with plasma protein and thus formation of drug plasma protein complex is often termed as protein binding. Different investigators have suggested that albumin has limited number of binding sites (Fehske *et al*., 1979; Hansen, 1981; Naher *et al*., 1997). On the basis of probe displacement method, it has been detected that there exist at least three relatively high affinity binding sites on BSA. These sites are commonly referred to as the warfarin, the benzodiazepine, and the digoxin site which are also denoted as site I, site II and site III, respectively (Fehske *et al*., 1981; Sudlow *et al*., 1975, 1976). Since the number of protein binding sites are limited, competition will exist between two drugs and the drugs with higher affinity will displace the other causing increased free drug concentration (Rahman, 1994).

Displacement of drug is defined as reduction in the extent of binding of a drug to protein caused by competition of another drug, the displacer. When two drugs that are capable of binding at the same sites on the protein are administered concurrently this type of competitive displacement is more likely. Pharmacokinetic drug interactions occur when one drug alter the absorption, distribution, metabolism, or excretion of another, thus increasing or reducing the amount of drug available to produce its pharmacological effects. In addition to other mechanisms, displacement of one drug from its binding site on plasma protein by another causes pharmacokinetic drug interaction (Kedderis, 1997; Hooper, 1999; BNF, 2003).

[+]*Correspondence*

Drug-drug interactions, more specifically, displacement interaction will affect the concentrations of free drugs in the plasma. Since the pharmacological activity of a drug is a function of free drug concentration, the displacement of even a small amount of drug bound to plasma protein could produce considerable increase in activity even leading to toxicity. The ability of one drug to inhibit the binding of the other is a function of their relative concentrations, binding affinities, and specificity of binding (Koch-Weser *et al*., 1976). However, when studying drug-drug displacement interactions, the possibility of displacement of drug from one site to another site should be taken into account. We have demonstrated this effect between dexamethasone phosphate and testosterone phenyl propionate (Rahman *et al*, 2001). Thus there will exist a difference between free concentration with or without such type of displacement from one binding site to another. Few studies have been carried out on what happens to the displaced fraction of a drug, when a protein bound drug is displaced from its binding site by a second drug. The purpose of the present study is to observe the competitive effect of ibuprofen on binding of naproxen and *vice versa* when used simultaneously. The study also aims at examining whether these drugs undergo site-to-site displacement when administered concurrently.

MATERIALS AND METHODS

Ibuprofen and naproxen were obtained from Rhone Poulence Rorer, Bangladesh Limited and Novartis (former

Ciba Geigy) Bangladesh Limited respectively. Ranitidine (site I blocking agent) was collected from Beximco Pharmaceuticals Ltd. and Sonear Laboratories Ltd. respectively. The solvents used (methanol, acetone, chloroform, ethanol) were of analytical grade and purchased from BDH, UK. The semi permeable membrane (Source: Union carbide Ltd.) with molecular cut off 6000 was purchased from local market. The pH meter (Lenway PHMIM) used was purchased from Jenway limited, UK. Pye Unicum double beam spectrophotometer (Model SP8-400 UV/VIS) was purchased from M/s Pye Unicum, Japan. Bovine serum albumin (BSA) was purchased as pure substance from Sigma Chemical Co. Ltd., St. Louis, U.S.A.Displacement of ibuprofen by naproxen and *vice versa* in the absence and presence of site specific probes when bound to BSA at pH 7.4 and 25°C was studied by equilibrium dialysis (Singlas, 1987) method.

Effect of ibuprofen on naproxen binding to BSA

In absence of ranitidine, site I specific probe: Five ml of $2x10^{-5}$ M BSA solution was taken in each of the six test tubes. Ten microlitre of $1x\ 10^{-2}$ M naproxen solution was added to each of the five test tubes to have the final naproxen to protein ratio at 1:1 ($2x10^{-5}$ M: $2x10^{-5}$ M). The sixth test tube containing only BSA solution was marked as 'control'. Ibuprofen solution was then added with increasing concentrations into four out of the five test tubes containing 1:1 naproxen-protein mixture to get ibuprofen concentration $0.2x10^{-5}$, $0.4x10^{-5}$, $0.6x10^{-5}$ and $2x10^{-5}$ M. The final ratios between ibuprofen to protein were 0.2:1, 0.4:1, 0.6:1, and 1:1. Ibuprofen was not added into the fifth test tube. Final volume of each tube was 5.02 ml. From each of the six test tubes 3.5 ml of solution was taken and poured into six different semipermeable membrane tubes. The tubes were then immersed in six separate 50-ml conical flasks containing 30 ml of phosphate buffer solution (pH 7.4). After proper mixing for about 30 minutes, they were placed in a metabolic shaker at 25°C. Shaking was continued for 12 hours. The concentrations of free naproxen were measured by a UV spectrophotometer at a wavelength of 331nm (BP 2000a).

In presence of ranitidine as site I blocking agent: Five ml of $2x10^{-5}$ M BSA solution was taken in each of the six test tubes. Twenty microlitre of $1x10^{-2}$ M ranitidine solution was added to each of the test tubes to make protein to ranitidine ratio 1:2 ($2x10^{-5}$ M: $4x10^{-5}$ M) to sufficiently block the site I on BSA. Then naproxen and ibuprofen were added, mixed properly, dialyzed and free concentration of naproxen was measured as mentioned above.

Effect of naproxen on ibuprofen binding to BSA

Similarly, effects of naproxen on ibuprofen binding to BSA both in absence and in presence of ranitidine hydrochloride were observed under similar experimental conditions. The concentrations of free ibuprofen were then measured by UV spectrophotometer at a wavelength of 264nm (BP 2000b).

Statistical method

Statistical analysis was carried out using Student *t* test with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

To study the possible interaction between two drugs identification of binding protein as well as binding sites of drugs on protein molecules are essential (Kober *et al.* 1980). Site-specific probe displacement method suggested that both ibuprofen and naproxen bind to site-II, the benzodiazepine site, with a higher affinity, while to site-I, the warfarin site, with relatively lower affinity. It was also found that the value of high affinity association constant for ibuprofen was greater than that of naproxen (M. M. Rahman, 1996). The free fraction of drug after equilibrium dialysis was calculated as follows:

Free fraction of naproxen or ibuprofen =

$$\frac{\text{Measured free concentration of naproxen or ibuprofen x100}}{\text{Concentration of naproxen or ibuprofen added}}\ (\%)$$

Drug-drug interactions

Fig.1 shows the changes in free concentrations of naproxen bound to BSA displaced by ibuprofen at pH 7.4 and 25°C both in the absence and presence of ranitidine (site I specific probe). Here ranitidine was used to sufficiently block the site I so that it is no more available to naproxen. The free concentration of naproxen bound to BSA, as shown in fig. 1, was increased from $2\pm0.1\%$ to $12\pm0.67\%$ by ibuprofen in the absence of ranitidine, whereas in the presence of ranitidine this increment was from $2\pm0.1\%$ to $18\pm1.0\%$ at the same ibuprofen concentration. The observation reveals that both ibuprofen and naproxen competes for the same binding site on BSA and naproxen is displaced from its high affinity binding site by ibuprofen. In absence of ranitidine, the increment in free concentration of naproxen by ibuprofen did not fit well with the theoretical curve as calculated from direct competition between ibuprofen and naproxen (not shown). Fig.1 shows that in presence of ranitidine, naproxen was displaced by ibuprofen to a greater extent from its binding site. Similarly, fig.2 shows the changes in free concentrations of ibuprofen displaced by naproxen both in the absence and presence of ranitidine. In the absence of ranitidine, the free concentration of ibuprofen was increased from $0.5\pm0.08\%$ to $0.8\pm0.12\%$ by naproxen, whereas in the presence of ranitidine this increment was from $0.5\pm0.08\%$ to $1.5\pm0.18\%$. The above results suggest that in presence of ranitidine, ibuprofen was

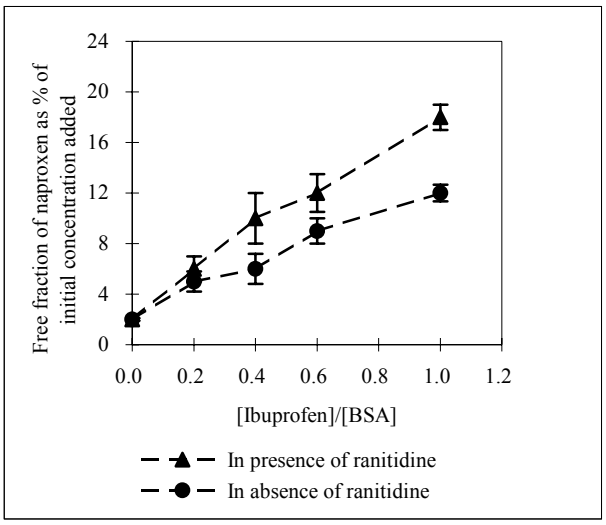


Fig.1 : Free fraction of naproxen as % of initial concen-tration added when bound to BSA (1:1) upon addition of ibuprofen in the presence (▲) and absence of ranitidine (●).
(Each values represent the average of 3 independent experiments ± SE)
Concentrations used:
[BSA]=[Naproxen]=2×10^{-5} M; [Ranitidine hydrochloride]= 4×10^{-5} M; [Ibuprofen]=0 - 2×10^{-5} M

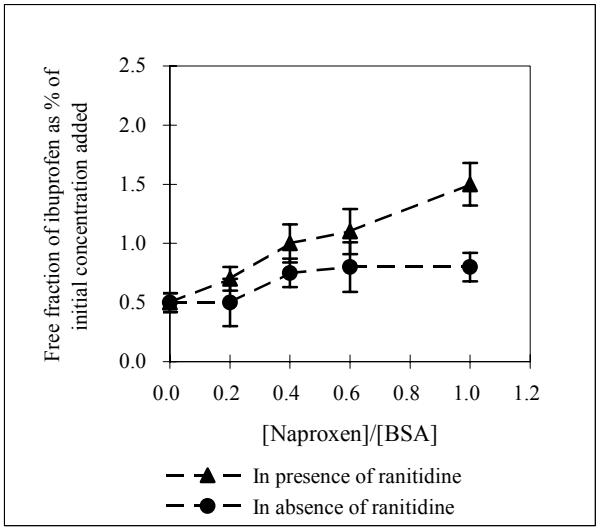


Fig.2: Free fraction of ibuprofen as % of initial concentration added when bound to BSA (1:1) upon addition of naproxen in the presence (▲) and absence of ranitidine (●).
(Each values represent the average of 3 independent experiments ±SE)
Concentrations used:
[BSA]=[Ibuprofen]=2×10^{-5} M; [Ranitidine hydrochloride]= 4×10^{-5} M; [Naproxen]=0 - 2×10^{-5} M

displaced by naproxen to a greater extent from its binding site. It was observed from the experiments that free concentration of naproxen by ibuprofen and *vice versa* was greater when site I was sufficiently blocked by ranitidine.

Both in the absence and presence of site I blocking agent, the extent of increment in free ibuprofen concentration by naproxen was less than that of naproxen by ibuprofen. This was supported by the fact that the value of high affinity association constant of ibuprofen ($3.25\text{x}10^6$ M^{-1}) is higher than that of naproxen ($1.31\text{x}10^6$ M^{-1}) (M. M. Rahman, 1996). That is, ibuprofen has greater affinity for its binding site on BSA than naproxen.

Proposed models

On the basis of the above results obtained during concurrent administration of ibuprofen and naproxen in the presence and absence of ranitidine, different models of drug-drug interaction have been proposed which are shown in figs. 3 and 4. Naproxen is displaced from its high affinity binding site (site-II) by ibuprofen and then a portion of the displaced drug rebound to its low affinity binding site (site-I) on the BSA molecule instead of remaining as free. This is why the free concentration of ibuprofen was not increased to a level as it was expected from direct competition (not shown). This was supported by the fact that when site I was sufficiently blocked by ranitidine, the free concentration of naproxen was further increased by the same amount of ibuprofen as the displaced naproxen could not undergo rebinding to site I. Although site II is the high affinity binding site for naproxen, in presence of ibuprofen, naproxen was displaced from site II and then rebound to site I. This modified form of drug-drug displacement with respect to their binding sites has been arbitrarily referred to as site-to-site displacement. The similar type of displacement pattern was observed when the free concentrations of ibuprofen were considered upon the addition of naproxen, both in the presence and absence of ranitidine (fig. 4).

Pharmacokinetic implications

During co-administration of ibuprofen and quinolones antibiotic in traumatized, stressed patients higher levels of the quinolones are obtained and adjustment of dosage is seriousle recommended (Trichilis *et al*, 2003). During concurrent administration of two or more drugs site-to-site displacement of one drug in the presence of another should be taken into consideration. Site–to-site displacement is very much important for drugs that remain highly protein bound and only a small free fraction is sufficient to cause pharmacological effect. There might be a remarkable difference between free drug concentration in presence and absence of site-to-site displacement. So care should exercised in calculation of free concentration of drugs that undergo site-to-site displacement as a small increase in free concentration of highly protein bound drug may augment therapeutic or toxic effect.

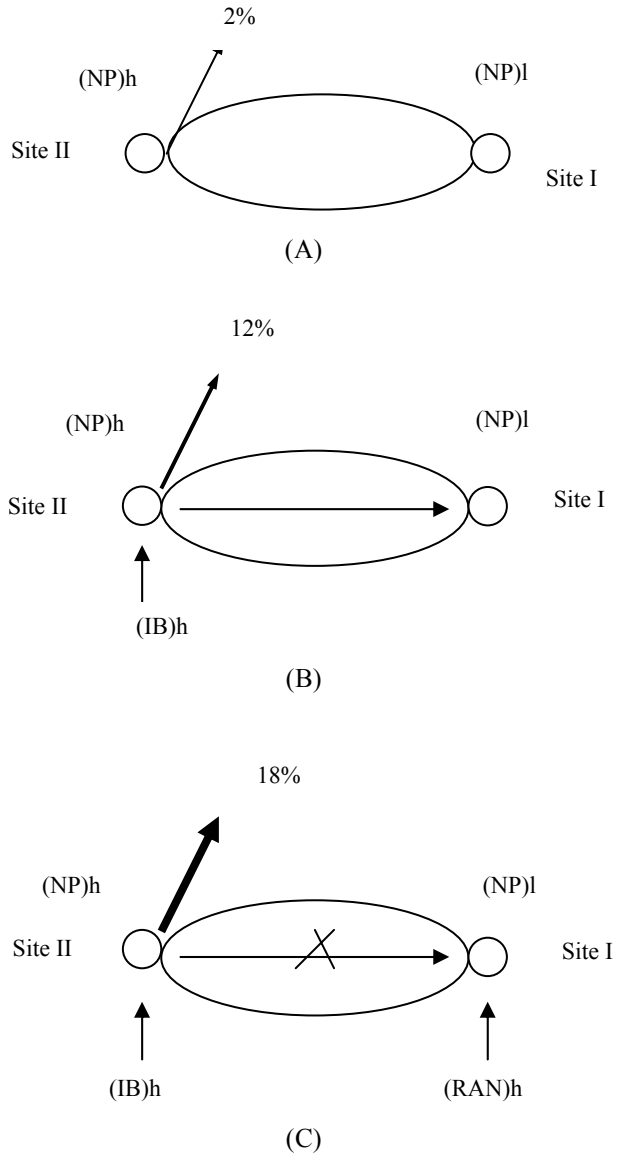
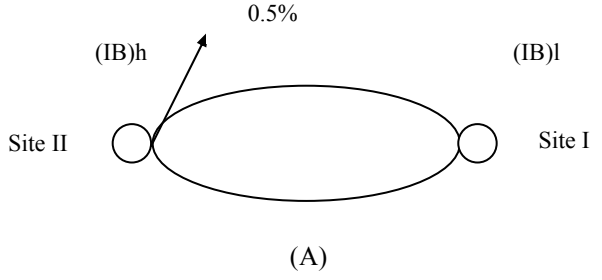


Fig. 3: Proposed models of the naproxen-BSA-ibuprofen interactions in the presence and absence of site I specific probe; NP, naproxen; IB, ibuprofen; RAN, ranitidine, a site I specific probe; h, high affinity; l, low affinity; (A) = normal binding of NP to BSA, (B) = effect of IB on NP bound to BSA in absence of RAN, (C) = effect of IB on NP bound to BSA in presence of RAN.



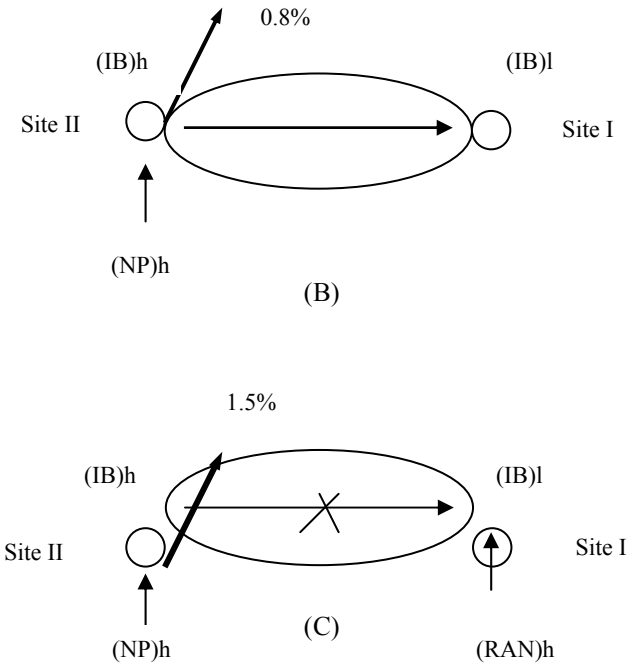


Fig. 4: Proposed models of the ibuprofen-BSA-naproxen interaction in the presence and absence of site I specific probe; IB, ibuprofen; NP, naproxen; RAN, ranitidine, a site I specific probe, h, high affinity; l, low affinity; (A) = normal binding of IB to BSA, (B) = effect of NP on IB bound to BSA in absence of RAN, (C)= effect of NP on IB bound to BSA in presence of RAN.

CONCLUSION

During concurrent administration of ibuprofen and naproxen, both the drugs compete for the same binding site on the albumin molecule. This results in remarkable increase in the free concentration of one drug by another on the basis of direct competitive displacement. However, because of so called site-to-site displacement it does not take place up to the expected theoretical extent. The initiation and intensity of pharmacological response and other pharmacokinetic properties of a drug is the function of its free plasma concentration. So, from the pharmacological and pharmacokinetic viewpoint, the possibility of direct competition for the same binding site as well as the concept site-to-site displacement should be brought into consideration when calculating dose of a drug during concurrent administration (like ibuprofen and naproxen).

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COMPARATIVE STUDY OF DIFFERENT FORMULATIONS OF ATENOLOL

RIFFAT YASMEEN, M. HARRIS SHOAIB AND HALIMA KHALID
Department of Pharmaceutics, Faculty of Pharmacy
University of Karachi, Karachi-75270, Pakistan

Tablets are the most common dosage form. Tablets can be prepared by dry method and wet methods, both methods have their own significance as well as disadvantages. Dry method and particularly *direct compression* is most simplest method of tablet manufacturing. On the other hand granulation is a multiple processing method which add complexity and make validation and control difficult.
For comparative study of atenolol tablets a new formulation was designed and compressed by *direct compression* method. Then it's physical parameters including hardness, friability, diameter, thickness, disintegration time, dissolution test were performed and finally assay carried out for evaluation and characterization of this new formulation against other formulation available in the market.

Keywords: Direct compression, wet granulation, atenolol, physical parameters.