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Binding of Dexamethasone Phosphate and Testosterone Phenyl Propionate to Bovine Serum Albumin: Drug–drug Interaction at the Binding Site

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Abstract: The binding of dexamethasone phosphate (DP) and testosterone phenyl propionate, (TPP) two semisynthetic steroids, to bovine serum albumin (BSA) was studied by equilibrium dialysis method at 25°C and pH 7.4 with a view to have an insight into the competitive binding characteristics of these two drugs, when bound to BSA simultaneously. There was increase in free concentration of DP due to addition of TPP and *vice versa* during concurrent administration of these two drugs, thereby causing reduced binding of these two drugs to BSA. However, the free fraction was not increased up to a level as it was expected from direct competitive displacement. In absence of the site I specific probe (warfarin sodium), DP after being displaced by TPP or *vice versa* from its high affinity binding site (site II) rebound to its low affinity binding site (site I) on BSA. However, when the site I was sufficiently blocked by warfarin, the increment in the free concentration of the displaced drug was more prominent. This form of modified displacement has been referred to as site-to-site displacement.

Key words: Equilibrium dialysis, bovine serum albumin, DP and TPP.

Introduction

The formation of drug plasma protein complex is often termed as drug plasma protein binding. Serum albumin, the most abundant protein in blood, plays a very important role in the binding phenomenon and serves as a depot protein and transport protein for numerous endogenous and exogenous compounds (Krag-Hansen, 1981). From different investigations, it has been suggested that albumin (human serum albumin, HSA) 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 HSA. 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 leading to increased toxicity (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 are capable of binding at the same sites on the protein, this type of competitive displacement is more significant. 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). Drug-drug interactions, more specifically, displacement interaction will affect the free concentrations of drugs in the blood. Since the pharmacologic 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. So, when studying drug-drug displacement interactions, the possibility of displacement of drug from one site to another site should be taken into account. Thus there will be a difference between free concentration with or without such displacement. The purpose of the present study is to observe the effect of Testosterone Phenyl Propionate (TPP) on free concentration of Dexamethasone Phosphate (DP) and *vice versa*.

Materials and Methods

DP and TPP were supplied by Organon (Bangladesh) Limited and site specific probe (warfarin sodium) was supplied by Gaco Pharmaceuticals Limited, Bangladesh. Dialysis membrane was purchased from Medicell International Limited, 239 Liverpool Road, London and BSA from the Sigma Chemical Co. Ltd.

Displacement of DP by TPP and *vice versa* in absence and presence of site specific probe when bound to BSA at 25°C and pH 7.4 was studied by equilibrium dialysis method (Singlas, 1987).

Effect of TPP on DP binding to BSA:

In absence of warfarin sodium, site I specific probe: Five ml of $2x\ 10^{-5}$ M BSA solution was taken in each of the six test tubes. Ten microlitre of $1x\ 10^{-2}$ M DP solution was added to each of the five test tubes to have the final dexamethasone and protein ratio at 1:1 ($2x\ 10^{-5}$ M: $2x\ 10^{-5}$ M) in each tube. The sixth test tube containing only BSA solution was marked as 'control'. TPP solution was then added with increasing concentrations into four out of the five test tubes containing 1: 1 mixture of protein and dexamethasone. The final ratios between testosterone phenyl propionate and protein were 1:1, 3:1, 5:1 and 7:1. TPP was not added into the fifth test tube containing dexamethasone–protein mixture (1:1). 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 20 ml of phosphate buffer solution (pH 7.4). After proper mixing they were placed in a metabolic shaker at 25 °C. Then shaking was continued for 10 hours. The free concentrations of DP were measured by a UV spectrophotometer (Spectronic, Genesys^{TM} 2, U.S.A.) at a wavelength of 242nm.

In presence of warfarin sodium: Five ml of $2x\ 10^{-5}$ M BSA solution was taken in each of the six test tubes. Twenty microlitre of $1x\ 10^{-2}$ M warfarin sodium solution was added to the test tubes so that the final ratio between protein and warfarin was 1:2 ($2x\ 10^{-5}$ M: $4x\ 10^{-5}$ M). Then ten microlitre of $1x\ 10^{-2}$ M DP solution was added to each of the five test

tubes to have the final ratio among protein, dexamethasone and warfarin sodium was 1:1:2 (2x 10^{-5} M: 2x 10^{-5} M: 4x 10^{-5} M) in each test tube. The sixth test tube containing only BSA solution and warfarin sodium was marked as 'blank'. Testosterone phenyl propionate solution was then added with increasing concentrations into four out of the five test tubes containing 1:1:2 mixture of protein, dexamethasone and warfarin. The final ratios between testosterone phenyl propionate and protein were 1:1, 3:1, 5:1 and 7:1. Testosterone phenyl propionate was not added into the fifth test tube containing protein-dexamethasone-warfarin mixture (1:1:2). 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 20 ml of phosphate buffer solution (pH 7.4). After proper mixing and shaking at 25°C for 10 hours in a metabolic shaker, free concentrations of dexamethasone phosphate were measured by a UV spectrophotometer (Spectronic, Genesys™ 2, U.S.A.) at a wavelength of 242nm.

Effect of DP on TPP binding to BSA: Similarly, effects of dexamethasone on testosterone phenyl propionate binding to BSA both in the absence and presence of warfarin sodium (site I specific probe) were observed under similar experimental conditions. The free concentrations of TPP were then measured by a UV spectrophotometer at a wavelength of 237nm.

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).

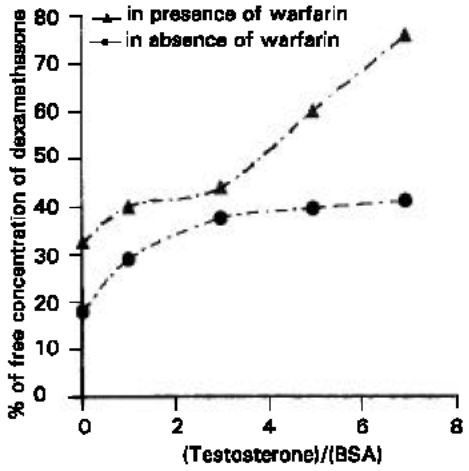

Fig. 1: Free fraction of dexamethasone phosphate as % of initial bound to BSA (1:1) upon the addition of testosterone phenyl propionate in the absence (●) and in the presence (▲) of warfarin sodium. Concentrations used:
[BSA] = [dexamethasone phosphate] = 2 x 10^{-5} M
[warfarin sodium] = 4 x 10^{-5} M, [diazepam] = 4 x 10^{-5} M
[testosterone phenyl propionate] = 0- 14 x 10^{-5} M

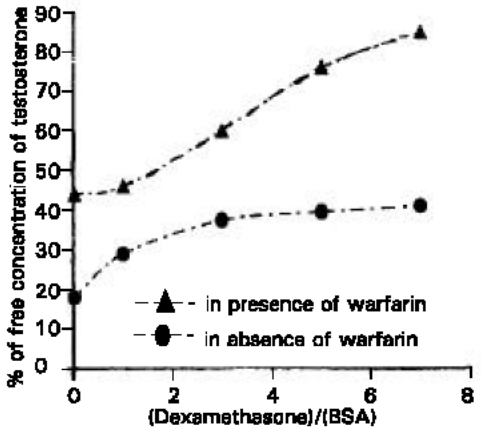

Fig. 2: Free fraction of testosterone phenyl propionate as % of initial bound to BSA (1:1) upon the addition of dexamethasone phosphate in the absence (●) and presence (▲) of warfarin sodium. Concentrations used:
[BSA] = [testosterone phenyl propionate] = 2 x10^{-5} M
[warfarin sodium] = 4 x 10^{-5} M,
[diazepam] = 4 x 10^{-5} M
[dexamethasone phosphate] = 0 - 14 x 10^{-5} M

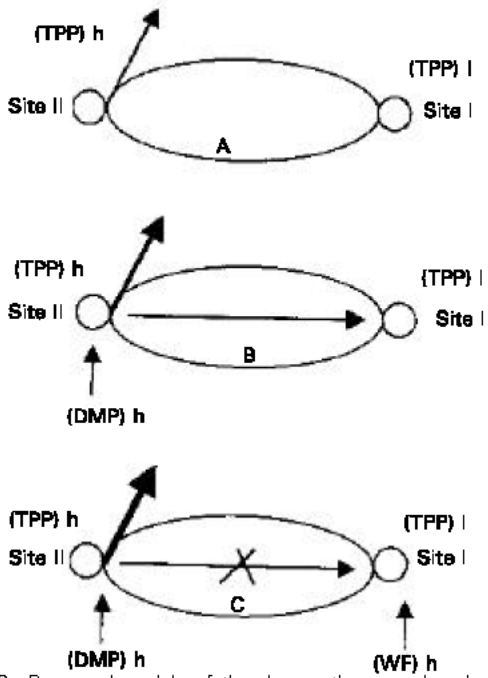

Fig. 3: Proposed models of the dexamethasone phosphate-BSA-testosterone phenyl propionate interactions in the presence and absence of site I specific probe; DMP, dexamethasone phosphate; TPP, testosterone phenyl propionate; WF, warfarin sodium; a site-I specific probe; h, high affinity; l, low affinity; (A) = normal binding of DMP to BSA, (B) = effect of TPP on DMP bound to BSA in absence of WF, (C)= effect of TPP on DMP bound to BSA in presence of WF.

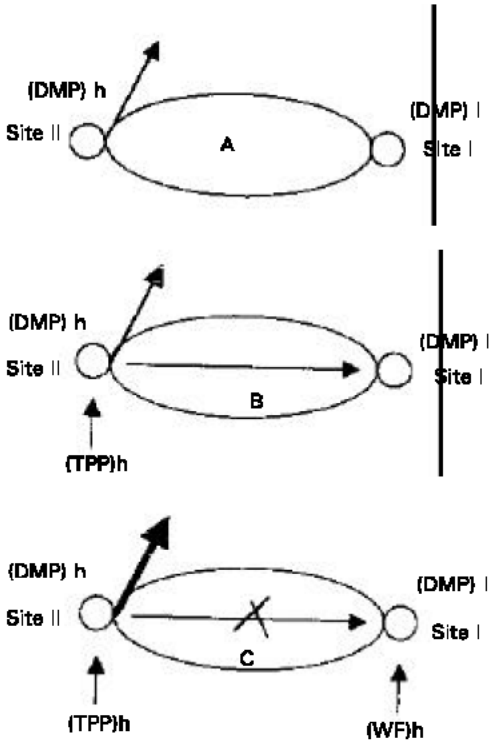

Fig. 4: Proposed models of the testosterone phenyl propionate-BSA-dexamethasone phosphate interactions in the presence and absence of site I specific probe; TPP, testosterone phenyl propionate; DMP, dexamethasone phosphate; WF, warfarin sodium, a site-I specific probe; h, high affinity; l, low affinity; (A) = normal binding of TPP to BSA, (B) = effect of DMP on TPP bound to BSA in absence of WF, (C) = effect of DMP on TPP bound to BSA in presence of WF.

Drug-drug interaction: Figure 1 shows the changes in free concentrations of DP bound to BSA displaced by TPP at pH 7.4 and 25 °C both in the absence and presence of warfarin sodium (site I specific probe). It is evident in Figure 1 that the free concentration of dexamethasone phosphate bound to BSA was increased from 18% to 41% by TPP in the absence of warfarin sodium, whereas in the presence of warfarin sodium this increment was from 32.5% to 76%. This suggested that in presence of warfarin sodium, DP was displaced to a greater extent from its binding site by TPP.

Similarly, Figure 2 shows the changes in free concentrations of TPP bound to BSA displaced by DP at pH 7.4 and 25°C both in the absence and presence of warfarin sodium. In the absence of warfarin sodium, the free concentration of TPP bound BSA was increased from 34% to 68% by DP , whereas in the presence of warfarin sodium this increment was from 44% to 85%. This suggested that in presence of warfarin sodium, TPP was displaced to a greater extent from its binding site by DP .

As observed from the experiments the free concentration of dexamethasone by testosterone and *vice versa* was higher in the presence of site I specific probes than that obtained in the absence of such probe.

Proposed models: On the basis of the above results obtained during concurrent administration of DP and TPP in the presence and absence of warfarin sodium, different models of drug-drug interaction have been proposed in Figures 3 and 4. DP is displaced from its high affinity binding site (site-II) by TPP and then a significant 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 DP was not increased to a level as it was expected from direct competition. However, when site I was sufficiently blocked by warfarin sodium, the free concentration of DP was increased further by the same amount of TPP. This modified form of drug-drug displacement has been arbitrarily referred to as site-to-site displacement. The similar type of displacement pattern was observed when the free concentrations of TPP were considered upon the addition of DP , both in the presence and absence of warfarin sodium.

Pharmacokinetic Implications: Plasma free drug concentration is one of the primary determinants of pharmacokinetic or pharmacodynamic properties of a drug. 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. So, care should exercised in calculation of free concentration of drugs that undergo site-to-site displacement.

Conclusion: During concurrent administration of Dexamethasone Phosphate and testosterone phenyl propionate, both the drugs compete for the same binding site on the albumin molecule. Thus, there should be a significant increase in the free concentration of both the drugs on the basis of direct competitive displacement. However, because of so called site-to-site displacement it does not take place. The initiation and intensity of pharmacologic response of a drug is also related to its free concentration. So, from the pharmacologic and pharmacodynamic view point, the concept site-to-site displacement should be brought into consideration when calculating dose of a drug during concurrent administration of two drugs (like DP and TPP).

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