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Research Article

EVALUATION OF *IN VITRO* INTERACTIONS OF WARFARIN AND DULOXETINE WITH SELECTED COADMINISTERED NSAIDS IN BOVINE SERUM ALBUMIN

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ABSTRACT

Numerous clinically significant drug interactions have been reported in the medical literature between Warfarin and nonsteroidal anti-inflammatory drugs (NSAIDs). The degree of binding of drugs to plasma protein has marked effect on its pharmacological effect. The binding of Warfarin and Duloxetine to bovine serum albumin in presence of selected NSAIDs (Aceclofenac, Diclofenac, Mefenamic acid and Naproxen) were studied by equilibrium dialysis method in order to understand the displacement interaction between these drugs. Results showed that the free (unbound) fraction of both Warfarin and Duloxetine were increased by all the selected NSAIDs. The magnitude of inhibition of protein binding of both Warfarin and Duloxetine by NSAIDs were as follows: Mefenamic acid>Diclofenac>Aceclofenac>Naproxen. Since Warfarin is a drug with low therapeutic index and low clearance, avoiding the concomitant use of Warfarin with these NSAIDs is the best way to prevent serious drug interactions. While this is not always possible, alternative therapies for either Warfarin or the NSAIDs must be considered. If the combination of Warfarin and NSAIDs therapy is necessary, it is important that patients be monitored closely (especially in the first few days) for changes in the level of their anticoagulation activity, with adjustment in the dose of Warfarin if necessary. Transient increase in free concentration of Duloxetine during concurrent administration with NSAIDs may not be clinically relevant since Duloxetine is a drug with extraction value higher than 90%.

Keywords: Warfarin, Duloxetine, Nonsteroidal Anti-inflammatory drugs, Bovine serum albumin, Protein binding, drug displacement interaction.

INTRODUCTION

The association of drugs with plasma protein and thus formation of drug plasma protein complex is often termed as protein binding[1]. 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. Competitive displacement interaction occurs when two drugs that are capable of binding at the same sites on the protein are administered concurrently[2]. Since the pharmacological activity of a drug is a function of free drug concentrations, the displacement of even a small amount of drug bound to plasma protein could produce considerable increase in activity, even leading to toxicity. This effect of protein binding is most significant with drugs that are highly protein-bound (>95%) and have a low therapeutic index, such as Warfarin. A low therapeutic index indicates that there is a high risk of toxicity when using the drug[3]. Since Warfarin is an anticoagulant with a low therapeutic index, it may cause bleeding if the correct degree of pharmacologic effect is not maintained. If a patient on Warfarin takes another drug that displaces Warfarin from plasma protein, it could result in an increased risk of bleeding[4]. A clinically significant interaction occurs for low clearance drugs with a low therapeutic index and a small V. The temporary increase in drug concentration and the increased variation within a dosing interval might be clinically relevant for such drugs. The drug best fitting this description is Warfarin (fu = 0.01, V= 91)[5]. Duloxetine is a novel antidepressant for which reports on displacement interactions are limited. Therefore, the aims of the present study were to understand the in-vitro displacement interaction between Warfarin/Duloxetine with selected nonsteroidal anti-inflammatory drugs like Aceclofenac, Diclofenac, Mefenamic acid and Naproxen.

MATERIALS AND METHODS

Dialysis membrane-110 (Av.diameter -21.5mm, capacity-3.63ml/cm) and free BSA (fraction V) were purchased from Himedia Laboratories, Pvt Ltd, Mumbai. Aceclofenac, Diclofenac, Mefenamic acid were obtained from Zydus Cadila. Warfarin, Naproxen, and duloxetin were free gift samples from Sun Pharma. All reagents used were analytical grade.

Displacement interaction study of Warfarin/Duloxetine with selected NSAIDs (Aceclofenac, Diclofenac, Mefenamic acid, and Naproxen) was conducted in two steps.

Protein binding of Warfarin/Duloxetine by Equilibrium Dialysis Method

Plasma protein binding was determined by equilibrium dialysis cell containing two chambers separated by a semi permeable membrane (Sigma dialysis)[6]. The dialysis membrane was previously activated by the following procedure. The membrane was immersed in boiling 1M $NaHCO_3$ solution for 1hour, then immersed in boiling demineralized water for about 1hour and finally immersed in phosphate buffer of pH 7.4 at about 70° C for 1 hour. 25ml of 1.5 x 10^{-4}M bovine serum albumin in phosphate buffer of pH 7.4 was taken inside the semi permeable membrane and 25ml of 6.72 x 10^{-4}M Warfarin in phosphate buffer (pH 7.4) was taken outside the semi permeable membrane. At zero time, 5ml of the solution was withdrawn from outside the semi permeable membrane and replaced with 5ml of the phosphate buffer. After making appropriate dilutions, absorbance was noted at 308 nm using a UV- visible spectrophotometer. Similar readings are taken at 30mins, 1hr, 1.5hrs, 2hrs, 2.5hrs, 3hrs, 3.5hrs, 4hrs, 4.5hrs, and 5hrs. Absorbance became constant at 5hrs, indicating that protein molecules are saturated with the drug at 5hrs. Concentration of the drug outside the membrane at different time intervals were determined using a previously prepared standard graph.

Effect of selected NSAIDs on Warfarin/Duloxetine binding to BSA

25ml of 1.5 x 10^{-4}M bovine serum albumin solution (BSA) was taken in each of 9 glass tubes attached to semi permeable membrane. The dialysis-membranes were previously activated. The tubes were then immersed in 9 separate 50 ml beakers containing a mixture of Warfarin and Aceclofenac in 25 ml of phosphate buffer of pH 7.4. The final ratio between Warfarin and Aceclofenac were 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8. The first glass tube contained only Warfarin of concentration 1.5 x 10^{-4}M. The system was maintained at room temperature for 5 hours. After 5 hours, 1ml of the solution was withdrawn from the beaker, diluted to 5 ml using potassium dihydrogen phosphate buffer and the concentration of free Warfarin were measured by a UV spectrophotometer at a wavelength of 308 nm. Similar steps were followed using Diclofenac/ Mefenamic acid/ Naproxen instead of Aceclofenac to find out their interaction with Warfarin. The displacement interaction between Duloxetine and NSAIDs were also found by the above mentioned procedure at 290 nm.

Data analysis: The percentage of protein binding of Warfarin/Duloxetine was estimated by using the formula, $(c_t - c_u) / c_t$ x 100

where, c_t = Total concentration of Warfarin / Duloxetine (in mcg/ml)

c_u = Concentration of unbound or free Warfarin / Duloxetine in mcg/ml.

Statistical analysis: Student's t-test was used to understand statistical significance. P-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In-vitro Interaction of Warfarin with BSA at various known concentrations is shown in table 1 and figure 1. Table 2 and figure 2 illustrate in-vitro interaction between Duloxetine and BSA.

Table 1: Interaction of Warfarin with BSA

S.No.	Total concentration of Warfarin in mcg/ml	Total concentration of Warfarin in M x 10^{-5}	Absorbance at 308nm	Concentration of unbound Warfarin in mcg/ml	Percentage protein binding	Percentage free drug concentration
1	1.65	0.5	0.43	1.28	22.75	77.91
2	3.30	1.0	0.86	2.14	35.14	63.6
3	4.96	1.5	0.94	2.64	46.75	51.65
4	6.61	2.0	1.08	2.67	59.62	39.86
5	8.26	2.5	0.99	2.64	68.04	29.58
6	9.91	3.0	0.93	2.58	74.0	20.64
7	11.56	3.5	0.58	1.55	86.5	13.5
8	13.21	4.0	0.44	1.31	90.12	9.88
9	14.86	4.5	0.37	1.01	93.2	6.8
10	16.52	5.0	0.18	0.57	96.56	3.44

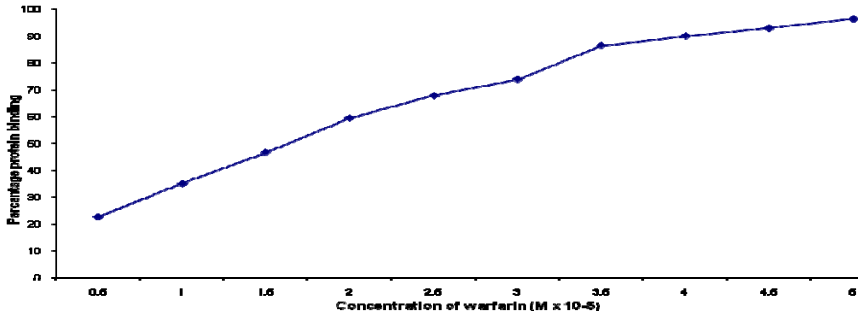

Fig. 1: Interaction of Warfarin with BSA

Table 2: Interaction of Duloxetine with BSA

S.No.	Total concentration of Duloxetine in mcg/ml	Total concentration of Duloxetine in M x 10^{-5}	Absorbance at 290 nm	Concentration of unbound Duloxetine in mcg/ml	Percentage protein binding	Percentage free drug concentration
1	1.49	0.5	0.40	1.29	12.79	87.21
2	2.98	1.0	0.92	2.51	15.74	84.26
3	4.46	1.5	1.16	3.19	28.36	71.64
4	5.95	2.0	1.29	3.79	36.24	63.76
5	7.44	2.5	1.32	3.93	47.11	52.89
6	8.92	3.0	1.16	3.30	63.01	36.99
7	10.40	3.5	0.91	2.54	75.6	24.4
8	11.89	4.0	0.54	1.97	83.4	16.6
9	13.38	4.5	0.45	1.29	90.3	9.7
10	14.87	5.0	0.22	0.86	94.2	5.8

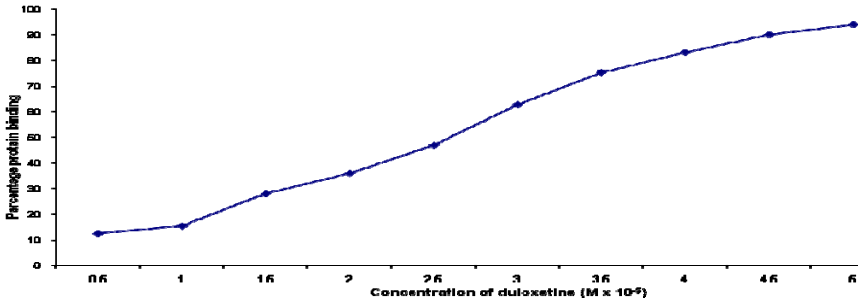

Fig. 2: Interaction of duloxetine with BSA

At low concentrations, the percentage of protein binding increases with increase in concentration of the drug. But at higher concentration, the percentage attains steady plateau, indicating the saturation zone for the binding of Warfarin/Duloxetine to BSA. In the present study the percentage of binding of Warfarin and Duloxetine to BSA at saturation level was 96.56% and 94.2% respectively.

Table 3 shows the data of in vitro displacement interaction between Warfarin and Aceclofenac/ Diclofenac/ Mefenamic acid/ Naproxen in a ratio of 1:0 to 1:8. The highest percentage of protein binding of Warfarin at saturation level was about 82.2% in presence of Aceclofenac, 80.7% in presence of Diclofenac, 68.2% in presence of Mefenamic acid, and 66.7% in presence of Naproxen.

By comparing this with Warfarin alone, it can be inferred that all the selected NSAIDs have significant effect on the protein binding of Warfarin. This may be due to good affinity of the NSAIDs for the protein. Figure 3 shows free fraction of Warfarin in presence of selected coadministered NSAIDs.

Table 3: Effect of NSAIDs on Warfarin binding to BSA (Equilibrium period = 5 hrs)

Sl. No.	Ratio of Warfarin : NSAIDs	Initial concentration of Warfarin ($M \times 10^{-4}$)	Concentration of NSAIDS ($M \times 10^{-4}$)	Percentage protein binding of Warfarin				Percentage free concentration of Warfarin			
				Aceclofenac	Diclofenac	Mefenamic acid	Naproxen	Aceclofenac	Diclofenac	Mefenamic acid	Naproxen
1	1:0	1.5	0	96.56	96.56	96.56	96.56	3.44	3.44	3.44	3.44
2	1:1	1.5	1.5	94.24	93.32	92.41	95.32	5.76	6.68	7.59	4.68
3	1:2	1.5	3.0	92.62	91.76	87.75	93.14	7.38	8.24	12.45	6.86
4	1:3	1.5	4.5	90.37	90.58	84.93	92.79	9.68	9.42	15.07	7.21
5	1:4	1.5	6.0	88.25	88.21	81.66	90.32	11.75	11.79	18.34	9.68
6	1:5	1.5	7.5	86.46	86.43	76.21	89.17	13.54	13.57	23.79	10.83
7	1:6	1.5	9.0	84.23	83.26	74.36	88.34	15.77	16.74	25.64	11.66
8	1:7	1.5	10.5	83.66	81.15	72.78	87.01	16.34	18.85	27.22	12.99
9	1:8	1.5	12.0	82.2	80.73	68.26	86.66	17.8 P = 0.279	19.27 P= 0.188	31.74 P= 0.014	13.34

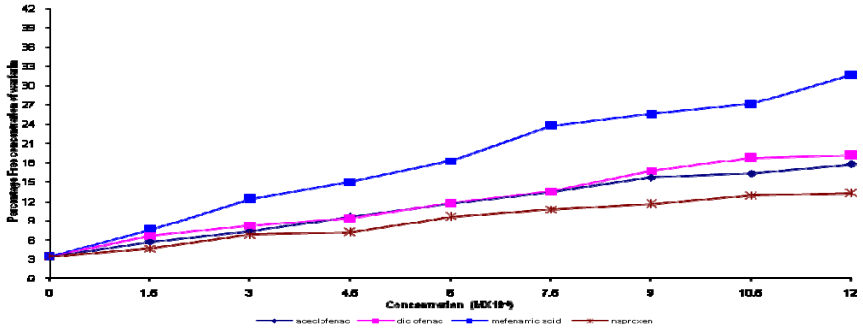

Fig. 3: Free fraction of warfarin in presence of coadministered nsaids

Table 4: Effect of NSAIDs on Duloxetine binding to BSA (Equilibrium period= 5hrs)

S. No.	Ratio of Duloxetine : NSAIDs	Initial concentration of Duloxetine ($M \times 10^{-4}$)	Concentration of NSAIDS ($M \times 10^{-4}$)	Percentage protein binding of Duloxetine				Percentage free concentration of Duloxetine			
				Aceclofenac	Diclofenac	Mefenamic acid	Naproxen	Aceclofenac	Diclofenac	Mefenamic acid	Naproxen
1	1:0	1.5	0	94.2	94.2	94.2	94.2	5.8	5.8	5.8	5.8
2	1:1	1.5	1.5	93.8	93.01	90.25	94.0	6.2	6.99	9.75	6.0
3	1:2	1.5	3.0	93.42	91.55	86.72	83.83	6.6	8.45	13.28	6.17
4	1:3	1.5	4.5	92.7	89.31	80.33	93.67	7.3	10.69	19.67	6.33
5	1:4	1.5	6.0	92.35	87.46	77.14	93.35	7.7	12.54	22.86	6.65
6	1:5	1.5	7.5	92.0	85.22	71.46	93.04	8.0	14.78	28.54	6.96
7	1:6	1.5	9.0	91.8	83.73	67.83	92.86	8.2	16.27	32.17	7.14
8	1:7	1.5	10.5	91.3	81.02	64.54	92.46	8.7	18.98	35.46	7.54
9	1:8	1.5	12.0	91.15	80.83	60.49	92.03	8.9	19.17	39.51	7.97

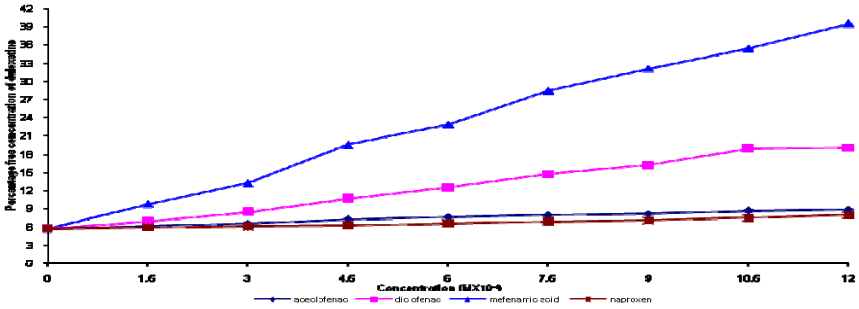

Fig. 4: Free fraction of duloxetine in presence of coadministered nsaids

Table 4 gives the data for in vitro interaction between Duloxetine and Aceclofenac/ Diclofenac/ Mefenamic acid/ Naproxen. Figure 4 shows free fraction of Duloxetine in presence of selected coadministered NSAIDs.

The highest percentage of protein binding of Duloxetine at saturation level was about 91.2% in presence of Aceclofenac, 80.83% in presence of Diclofenac, 60.49% in presence of Mefenamic acid, and 92.03% in presence of Naproxen. By comparing this with Duloxetine alone, it can be confirmed that all the selected NSAIDs have significant effect on the protein binding of Duloxetine.

The relative increase in the free fraction of Warfarin was 14.36% (P=0.279) in presence of Aceclofenac, 15.83% (P=0.188) in presence of Diclofenac, 28.26% (P=0.014) in presence of Mefenamic acid and 10.00% in presence of Naproxen. The relative increase in the free fraction of Duloxetine was 3.10% (P=0.103) in presence of Aceclofenac, 13.37% (P=0.003) in presence of Diclofenac, 33.7% (P=0.0008) in presence of Mefenamic acid and 2.17% in presence of Naproxen. The magnitude of inhibition of protein binding of both Warfarin and Duloxetine by NSAIDs were found in the order: Mefenamic acid > Diclofenac > Aceclofenac > Naproxen.

Literature suggests that a plasma protein binding displacement interaction may be clinically significant for low clearance drugs with a low therapeutic index and a small volume of distribution. For such drugs, the temporary increase in drug concentration and the increased variation within a dosing interval might be clinically relevant[7].

Present study demonstrated significant elevations in the level of unbound concentration of both Warfarin and Duloxetine in presence of selected NSAIDs. Co administration of Warfarin with these NSAIDs may not be safe as Warfarin is a drug with low therapeutic index, low clearance and small volume of distribution (0.11-0.2 L/kg). Increase in the plasma concentration of Warfarin may lead to bleeding[8]. Therefore, concomitant use of Warfarin and these NSAIDs must be avoided in order to prevent serious drug interactions. Alternative therapies must be considered whenever possible. If the combination of Warfarin and NSAID therapy is necessary, it is important that patients be monitored closely (especially in the first few days) for changes in the level of their anticoagulation activity, with adjustment in the dose of Warfarin if necessary. Duloxetine is a drug with extraction value higher than 90%. It is rapidly and extensively metabolized to form multiple oxidative and conjugated metabolites[9]. Therefore, a transient increase in free concentration of Duloxetine during concomitant administration with NSAIDs may not be clinically relevant.

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