COMPETITION BETWEEN COX-2 INHIBITORS AND SOME OTHER DRUGS FOR BINDING SITES ON HUMAN SERUM ALBUMIN

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SUMMARY

Competitive binding of six COX-2 inhibitors (celecoxib, valdecoxib, etoricoxib, parecoxib sodium, meloxicam and nimesulide) in the presence of three categories of drugs: an antidiabetic (gliclazide), antipsychotic (chlorpromazine) and antibiotic (ceftriaxone sodium) were studied by fluorescence spectroscopy. Data are expressed in terms of the percentage of drug bound in the absence and presence of competing drug and change in the percentage of free drug due to competitive binding. The results are discussed in terms of three cases: decrease in the binding of the parent drug; increase in binding; and no effect by the presence of the competing drug. The relative binding affinity of the parent drug and the displacing compound for human serum albumin (HSA) is not the only factor involved in the competitive mechanism. Binding behaviour of individual drugs was analysed, and explanations for different cases based on the competitive displacement, non-competitive interference, conformational changes in the HSA molecule, and independent binding are presented.

KEY WORDS

competitive binding, mechanism, COX-2 inhibitors, human serum albumin, fluorescence

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INTRODUCTION

The pharmacological activity of many drugs is altered by the presence of other drugs. Concomitantly administered drugs can compete with one another for binding sites on albumin (competitive interference) or they may cause conformational changes in the binding protein (non-competitive interference). Drugs may also bind independently at different sites in the human serum albumin (HSA) molecule with no conformational changes. The increase/decrease in the concentration of free drug due to competitive binding may result in enhanced pharmacological effect (toxicity) or reduced therapeutic efficacy. However, drug displacement interactions are clinically significant only for low clearance, highly protein bound drugs with small volume of distribution and a low therapeutic index /1/. Competitive binding of drugs to HSA has been reviewed by a number of investigators /2-4/. Studies on some NSAIDs have also been reported /5,6/. In the present study, competitive binding of six COX-2 inhibitors and three other drugs was studied by fluorescence spectroscopy. The results are expressed in terms of the change in the concentration of free pharmacologically active drug and the competitive mechanism involved.

MATERIALS AND METHODS

Competitive binding of six COX-2 inhibitors (celecoxib, valdecoxib, etoricoxib, parecoxib sodium, meloxicam and nimesulide) in the presence of three categories of drugs: an antidiabetic (gliclazide), antipsychotic (chlorpromazine) and antibiotic (ceftriaxone sodium), was studied by fluorescence spectroscopy. Eighteen drug combinations were studied. For each combination, the following four experiments were performed:

(i) HSA-drug 1

(ii) HSA-drug 2

(iii) HSA+drug 1-drug 2

Abbreviations: CEL = celecoxib; VLD = valdecoxib; ETR = etoricoxib; PRX = paracoxib sodium; MLX = meloxicam; NMD = nimesulide; CPZ = chlorpromazine hydrochloride; GLZ = gliclazide; CFZ = ceftriaxone sodium; HSA = human serum albumin.

(iv) HSA+drug 2-drug 1.

HSA was titrated with drug 1 in the absence and presence of drug 2. HSA was also titrated with drug 2 in the absence and presence of drug 1. For the binding of single drugs, HSA concentration was kept fixed (10 μM), and for the competitive binding studies, the concentration of an equimolar mixture of HSA+drug (10 μM each) was kept fixed. In each case the drug concentration was varied from 5-50 μM in the case of celecoxib, valdecoxib, etoricoxib, parecoxib sodium, chlorpromazine hydrochloride, gliclazide and ceftriaxone sodium, and 2-30 μM in the case of meloxicam and nimesulide. Fluorescence of HSA was recorded at 334 nm after excitation at 296 nm.

Binding data were analysed by the method standardized in our earlier publications /7-9/. Results were expressed as percentage of drug bound and percentage of free drug at different drug:protein ratios. Percentage of drug bound was calculated from fractional saturation,

$$\theta = \Delta F/\Delta F_{max},$$

where $\Delta F = F_0 - F$.

F_0 and F are the fluorescence intensities of HSA at 334 nm in the absence and presence of drug, respectively. ΔF_{max} values were obtained from the double reciprocal ($1/\Delta F$ versus $1/D_t$) plots. In the case of drug combinations, fluorescence of an equimolar mixture of HSA and one of the drugs was taken as F_0 and fluorescence intensity after the addition of increasing amounts of the other drug was taken as F.

The percentage of drug bound for single drugs as well as various drug combinations was calculated as follows: the total number of binding sites on protein = $n P_t$, concentration of bound sites on protein = $n \theta P_t$, where P_t is the total protein concentration, n is the number of binding sites and θ is the fractional saturation of protein binding sites. The value of n was determined by the method of continuous variations /10/. $n \theta P_t$ = concentration of bound drug (D_b). The percentage of drug bound, $\beta = D_b/D_t \times 100 = [n \theta P_t/D_t] \times 100$.

Since concentration of free drug $D_f = D_t - D_b$, the percentage of free drug, $\alpha = D_f/D_t \times 100$, was also calculated. Data were obtained for all the single drugs as well as various drug combinations.

The association constant (K_a) for the binding was computed directly by fitting the experimental data (r and D_f values) to the

following general equation (Scatchard equation) using an iterative non-linear least squares regression program developed for this purpose:

$$r = \sum_{i=1}^{i=m} n_i K_{ai} D_f / (1 + K_{ai} D_f) \quad (1)$$

RESULTS AND DISCUSSION

Competitive binding of 18 drug combinations, consisting of six COX-2 inhibitors and three other drugs: an antidiabetic (gliclazide), an antipsychotic (chlorpromazine hydrochloride) and an antibiotic (ceftriaxone sodium), was studied. All nine drugs are suitable candidates for competitive binding studies since they are more than 90% bound to plasma proteins, have a small volume of distribution and a low therapeutic index. In each combination, quenching of HSA fluorescence by drug 1 in the absence and presence of drug 2 and quenching of HSA fluorescence by drug 2 in the absence and presence of drug 1 were studied. The results, showing the effect of the presence of the competing drug on the binding of the parent (binding) drug, are summarized in Tables 1 and 2. The following three cases can be distinguished:

Case I: Binding of a given drug (binding drug) is decreased by the presence of the competing drug.

Case II: Binding of a given drug (binding drug) is increased by the presence of the competing drug.

Case III: Binding of a given drug (binding drug) is unaffected by the presence of the competing drug.

The data, expressed as percentage of drug bound [$\beta = (D_b/D_t) \times 100$], were plotted against drug:protein ratio (D_t/P_t) in Figures 1-5 for some representative single drugs and drug combinations.

The decrease in the degree of binding of the parent (binding) drug in the presence of the competing drug can be interpreted as the displacement of the parent drug from its binding sites on HSA /11/. The consequence of the decreased binding is an increase in the concentration of free drug. Competitive binding may also cause structural changes in the albumin molecule thereby creating more

TABLE 1

Effect of competing drugs on the percentage binding of various COX-2 inhibitors to human serum albumin

Competing drug [a]	Percentage binding					
	Binding drug [a]					
	Celecoxib	**Valdecoxib**	**Etoricoxib**	**Parecoxib**	**Meloxicam**	**Nimesulide**
Chlorpromazine HCl	Decrease (0.5-5.0) [b]	Increase (0.5-2.5) [b]	Increase (0.5-1.0) [b]	Increase (0.5-5.0) [b]	Increase (0.5-1.0) [b]	Decrease (0.5-3.0) [b]
Gliclazide	Decrease (0.5-3.0) [b]	Decrease (0.5-5.0) [b]	No effect	Increase (0.5-4.0) [b]	Decrease (0.5-5.0) [b]	Decrease (0.5-3.0) [b]
Ceftriaxone sodium	Decrease (0.5-1.5) [b]	Decrease (0.5-1.0) [b]	Increase (0.5-2.0) [b]	No effect	Increase (0.5-1.0) [b]	No effect

[a] Binding drug is the titrant. Binding of titrant is studied in the absence and presence of competing drug.

[b] Values in paretheses represent D_t/P_t range where the effect was observed for different combinations.

TABLE 2

Effect of various COX-2 inhibitors (competing drugs) on the percentage binding of other drugs to human serum albumin

Binding drug [a]	Percentage binding					
	Competing drug [a]					
	Celecoxib	Valdecoxib	Etoricoxib	Parecoxib	Meloxicam	Nimesulide
Chlorpromazine HCl	Decrease (0.5-3.5)	Increase (0.5-2.5)	Increase (0.5-5.0)	Increase (0.5-5.0)	Decrease (0.5-5.0)	Increase (0.5-4.0)
Gliclazide	No effect	Decrease (0.5-5.0)	Decrease (0.5-5.0)	Decrease (0.5-5.0)	Increase (0.5-2.0)	Decrease (0.5-1.0)
Ceftriaxone sodium	No effect	Decrease (0.5-2.0)	No effect	Decrease (0.5-1.5)	Decrease (0.5-2.0)	Decrease (0.5-1.5)

[a] Binding drug is the titrant. Binding of titrant is studied in the absence and presence of competing drugs.
[b] Values in paretheses represent D_t/P_t range where the effect was observed for different combinations.

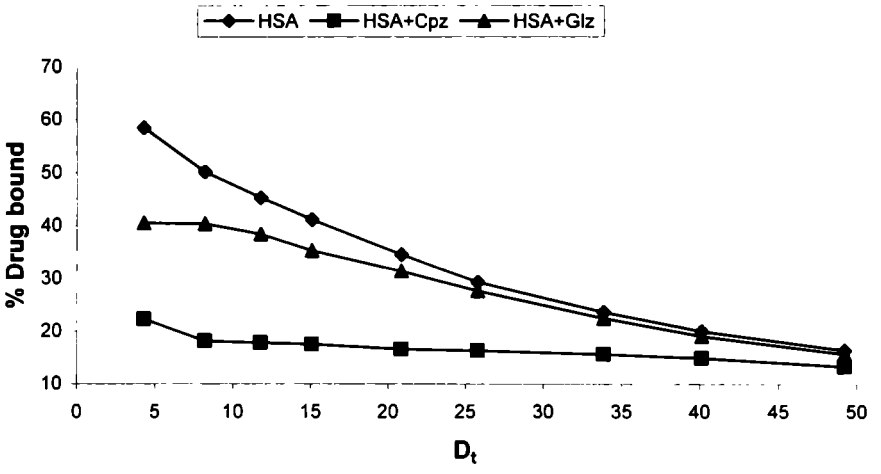

Fig. 1: Percentage celecoxib bound to human serum albumin (HSA) in the absence and presence of competing drugs.

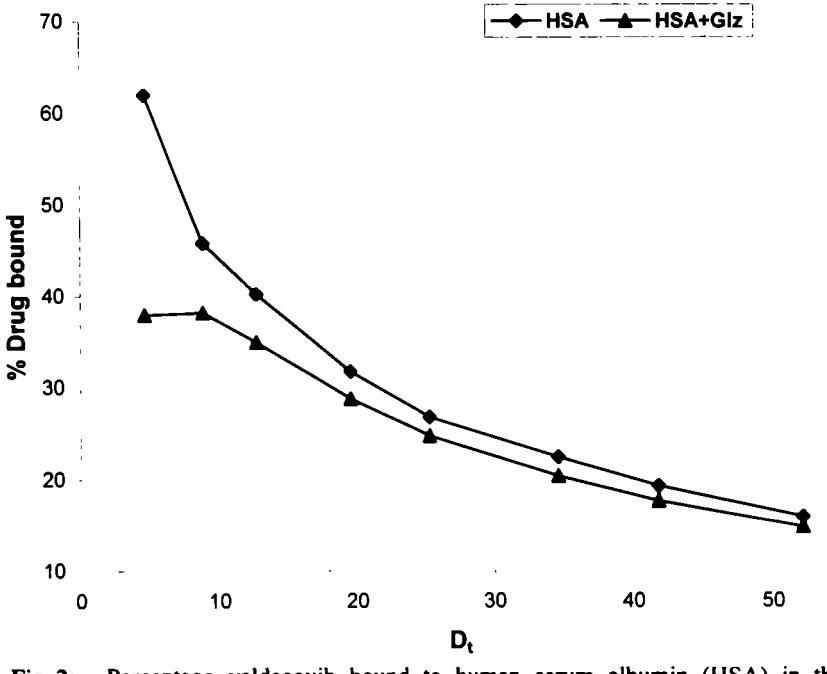

Fig. 2: Percentage valdecoxib bound to human serum albumin (HSA) in the absence and presence of competing drugs.

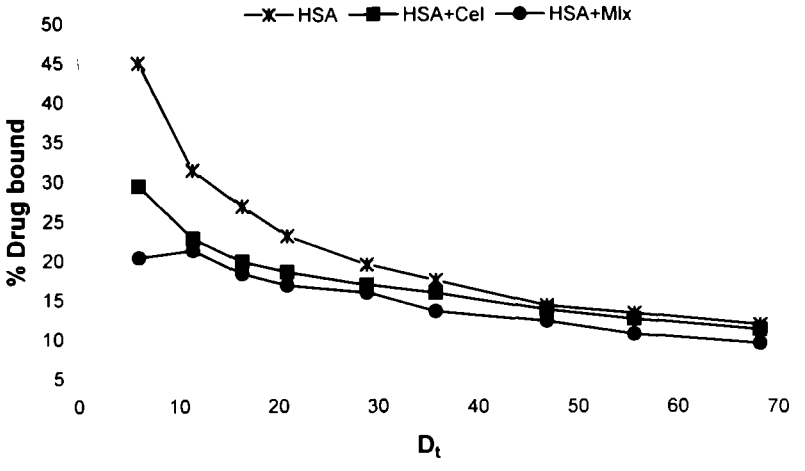

Fig. 3: Percentage of chlorpromazine bound to human serum albumin (HSA) in the absence and presence of competing drugs.

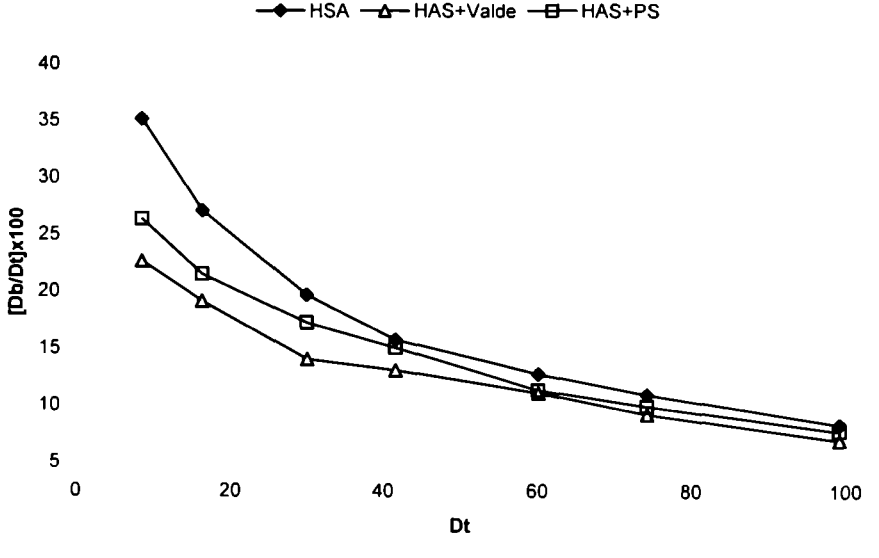

Fig. 4: Percentage of gliclazide bound to human serum albumin (HSA) in the absence and presence of competing drugs.

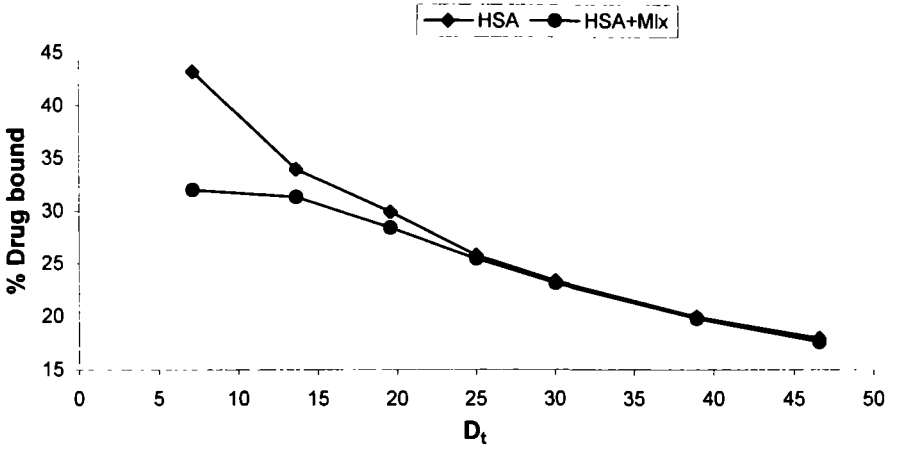

Fig. 5: Percentage of ceftriaxone sodium bound to human serum albumin (HSA) in the absence and presence of competing drugs.

binding sites or increasing the accessibility of the existing sites. Thus, the presence of the competing drug can also result in an increase in the degree of binding of the parent drug and consequently decrease in the concentration of free drug. If the two drugs bind independently, they probably bind at different sites in the HSA molecule and there are no conformational changes involved. In such a case, the binding of a given drug will not be affected by the presence of the competing drug.

Change in the percentage of free drug due to competitive binding

As discussed above, the alteration in the binding affinity of a given drug in the presence of competing drugs results in change in the concentration of free pharmacologically active drug. The change in the percentage of free drug due to competitive binding ($\Delta\alpha$) was calculated as $\Delta\alpha = (\Delta D_f/D_t) \times 100$, where $\Delta D_f = D_f^* - D_f$. D_f^* and D_f are the concentration of free drug in the presence and absence of competing drug, respectively. $\Delta\alpha$ values for various drug combinations were calculated over the D_t/P_t range 0.5-5.0 (data not given). The change in the percentage of free drug was found to be significant only at low drug:protein ratios. Low drug:protein ratios are especially important, since in the biological system the concentration of HSA is much higher than that of the drug. The change in the percentage of free drug at D_t/P_t ratio of 0.5 (the lowest drug:protein ratio studied), for all the

drug combinations, is given in Table 3. For the interpretation of the data, less than 5% change in the percentage of free drug, $\Delta\alpha = [(\Delta D_f/D_t)\ x100]$, was taken as negligible, while 5-10% was taken as a small change, and more than 10%, a significant change. Based on this criterion, the following inferences could be drawn:

1. Increase in the concentration of free drug

i) Significant increase (>10%): Significant increase in the concentration of free drug was observed in the following cases:

a. Binding of celecoxib in the presence of chlorpromazine and gliclazide.
b. Binding of valdecoxib in the presence of gliclazide.
c. Binding of chlorpromazine in the presence of celecoxib and meloxicam.
d. Binding of gliclazide in the presence of valdecoxib and parecoxib sodium.
e. Binding of ceftriaxone in the presence of meloxicam.

ii) Small increase (5-10%): Small increase in the concentration of free drug was observed in the following cases:

a. Binding of celecoxib in the presence of ceftriaxone.
b. Binding of nimesulide in the presence of chlorpromazine and gliclazide.
c. Binding of ceftriaxone in the presence of valdecoxib.

2. Decrease in the concentration of free drug

i) Significant decrease (>10%): Significant decrease in the concentration of free drug was observed only for the binding of meloxicam in the presence of ceftriaxone sodium.

ii) Small decrease (5-10%): A small decrease in the concentration of free drug was observed in the following cases:

a. Binding of valdecoxib, parecoxib sodium and meloxicam in the presence of chlorpromazine.
b. Binding of parecoxib sodium in the presence of gliclazide.
c. Binding of etoricoxib in the presence of ceftriaxone.
d. Binding of chlorpromazine in the presence of valdecoxib, parecoxib sodium, nimesulide and etoricoxib.

TABLE 3

Change in the percentage of free binding drug in the absence and presence of competing drug at drug:protein ratio of 0.5

Competing drug	Change in the percentage of free binding drug (α) at D_t/P_t ratio of 0.5								
	CEL	VLD	ETR	PRX	MLX	NMD	CPZ	GLZ	CFT
CEL		–	–	–	–	–	+14.2	NC	NC
VLD	–		–	–	–	–	-5.6	+17.8	+8.0
ETR	–	–		–	–	–	-8.0	+4.8	NC
PRX	–	–	–		–	–	-6.0	+14.8	+2.0
MLX	–	–	–	–		–	+18.6	-3.2	+12.0
NMD	–	–	–	–	–		-7.0	+2.0	+4.0
CPZ	+34.1	-5.2	-5.0	-5.2	-5.5	+9.5		–	–
GLZ	+15.1	+22.8	NC	-5.1	+2.5	+5.0	–		–
CFT	+8.0	+2.8	-4.9	NC	-11.4	NC	–	–	

Positive sign indicates increase in the concentration of free drug.
Negative sign indicates decrease in the concentration of free drug.
NC indicates no change in the concentration of free drug.
Dash (–) indicates combinations which have not been studied.

3. No change in the concentration of free drug

In the following systems, the binding of a given drug was unaffected by the binding of another drug:

a. Binding of valdecoxib, parecoxib sodium and nimesulide in the presence of ceftriaxone.
b. Binding of meloxicam and etoricoxib in the presence of gliclazide.
c. Binding of gliclazide in the presence of celecoxib, meloxicam, nimesulide and etoricoxib.
d. Binding of ceftriaxone in the presence of celecoxib, nimesulide, parecoxib sodium and etoricoxib.

Data for cases in which significant change in the concentration of free drug was observed are given in Table 4 for the entire D_t/P_t range studied (0.5-5.0).

Binding characteristics of the drugs used

For interpretation of the results of competitive binding studies, it is necessary to discuss the HSA binding behaviour of the individual drugs used. The binding constants of all nine drugs used in various combinations are given in Table 5. It is seen that the association constant data are not adequate to explain the competitive binding results discussed above. It thus appears that factors other than the relative binding affinities of the binding and competing drug are also involved. It has also been reported by others /12/ that the degree of displacement cannot always be predicted directly by consideration of the respective association constants of the drug and the displacing compound.

Site specificity of the COX-2 inhibitors used in the present study has already been reported /7-9/. All COX-2 drugs except nimesulide bind preferentially at site II. Nimesulide binds at the interface between site I and site II. Amongst the competing drugs, both chlorpromazine and gliclazide contain delocalized negative charge at the centre of a largely non-polar molecule, which is a characteristic of drugs binding to site I /13/. Fujii *et al.* /14/ have also shown that the binding of gliclazide decreases in the presence of phenylbutazone, a site I drug. Ceftriaxone, being a bulkier molecule, is also expected to bind at site I, which is larger and more flexible than site II. Displacement studies

TABLE 4

Change in the percentage of free drug due to competitive binding

D_t/P_t	Change in the percentage of free drug (Δα)								
	Cele		Vld	Cpz		Glz		Melx	Cft
	In the presence of								
	Cft	Glz	Glz	Cele	Melx	Vld	PS	Cft	Melx
0.5	+34.1	+15.1	+22.8	+14 2	+18.6	+17.8	+14.8	-11.4	+12.0
1.0	+28.2	+7.7	+8.0	+8.5	+12.5	+10.7	+7.4	-6.0	+7.0
1.5	+23.4	+7.0	+3.5	+6.0	+8.0	+8.7	+6.8	–	+1.5
2.0	+19.7	+4.5	+2.5	+5.6	+7.0	+8.0	+6.6	–	+0.8
2.5	+13.6	+1.5	+2.2	+3.2	+4.4	+6.5	+4.3	–	–
3.0	+9.8	+1.1	+2.1	+2.4	+3 9	+5.7	+2.6	–	–
3.5	+7.6	-	+2.0	+0.7	+3.4	+4.6	+1.8	–	–
4.0	+4.9	-	+2.0	-	+2.9	+3.9	+1.8	–	–
4.5	+3.8	-	+1.7	-	+2.7	+2.6	+1.3	–	–
5.0	+2.6	-	+1.6	-	+2.4	+2.3	+1.1	–	–

Positive sign indicates increase in the concentration of free drug.
Negative sign indicates decrease in the concentration of free drug.
Dash (–) indicates no change in the concentration of free drug

TABLE 5

Association constants for the binding of various COX-2 inhibitor drugs and competing drugs to HSA

Drug	Association constant* ($K_a \times 10^{-5}.M^{-1}$)
Celecoxib	0.502
Valdecoxib	1.272
Etoricoxib	0.308
Parecoxib sodium	0.552
Meloxicam	1.425
Nimesulide	1.495
Chlorpromazine HCl	0.789
Gliclazide	0.796
Ceftriaxone sodium	0.889

* Determined experimentally under identical conditions.

carried out by Tawara *et al.* /15/ have also shown that ceftriaxone binding to HSA is decreased in the presence of site I marker ligands but is unaffected by site II ligands. Thus, all the three competing drugs appear to bind preferentially at site I of HSA. Some physical properties of the binding and competing drugs are given in Table 6.

Competitive binding results

The binding behaviour of each drug in the presence of various competing drugs is discussed below:

Celecoxib: The binding of celecoxib was decreased in the presence of all three competing drugs, in the order chlorpromazine hydrochloride > gliclazide > ceftriaxone sodium. The effect was highly significant in the case of chlorpromazine where up to 34% increase in the concentration of free celecoxib was observed and least in the case of ceftriaxone sodium where the increase was

TABLE 6

Some physicochemical properties of the competing drugs

Competing drug	Properties*		
	log P	TPSA	Molar volume
Celecoxib	3.24	77.99	266.40
Valdecoxib	2.40	86.20	241.20
Etoricoxib	2.37	59.93	276.40
Parecoxib sodium	1.34	89.27	–
Meloxicam	3.87	99.60	217.70
Nimesulide	2.11	101.23	212.30
Chlorpromazine HCl	5.03	8.17	285.37
Gliclazide	1.45	78.50	284.59
Ceftriaxone sodium	-2.19	174.780	564.57

* Determined using software molinspiration.
TPSA = total polar surface area.

only 8%. Since celecoxib and the three competing drugs bind at different sites in the HSA molecule, the increase in the concentration of free celecoxib in the presence of these drugs cannot be attributed to competitive displacement. The mechanism of displacement appears to be non-competitive where the tendency of one drug to displace another depends on its ability to distort the albumin molecule. Silva *et al.* /16/ have shown that chlorpromazine alone also causes conformational changes in the albumin molecule. The large increase in the percentage of free celecoxib in the presence of chlorpromazine could be the result of the combined effect of celecoxib and chlorpromazine on the structure of albumin. The effect is smaller in the case of gliclazide since gliclazide alone does not cause structural changes in albumin. In the case of ceftriaxone sodium, the increase is not very significant (<10%). A comparison of the physicochemical properties of the three competing drugs (Table 6) shows that the hydrophobicity of the drugs, estimated from partition coefficients (log P) and total

polar surface area (TPSA), follows the same order (chlorpromazine hydrochloride > gliclazide > ceftriaxone sodium) as the displacing potential of these drugs. Thus hydrophobicity appears to be an important factor in the ability of the competing drug to displace celecoxib.

Valdecoxib: In the case of valdecoxib, the percentage of free drug showed a significant increase in the presence of gliclazide and a small decrease in the presence of chlorpromazine. Ceftriaxone, on the other hand, did not cause any change in the concentration of free valdecoxib. Since chlorpromazine causes conformational changes in the albumin molecule /16/, the small increase in the binding of valdecoxib in the presence of chlorpromazine at low D_t/P_t ratio could be due to the increase in the accessibility of the valdecoxib binding site in the presence of chlorpromazine and consequent decrease in the concentration of free valdecoxib. Gliclazide, however, displaces valdecoxib from HSA. Since gliclazide does not share common binding sites with valdecoxib and has lower binding affinity, it appears that non-competitive interference due to the simultaneous presence of both drugs is responsible for the displacement of valdecoxib. Ceftriaxone sodium, a significantly large and polar molecule compared to other drugs, had no effect on the binding of valdecoxib.

Parecoxib sodium: In the case of parecoxib sodium, again ceftriaxone sodium had no effect on binding, while chlorpromazine and gliclazide both increased the binding of parecoxib sodium. However, the change in free drug concentration was only 5% in both cases. Since these drugs do not share the same binding site with parecoxib sodium, the small increase in binding and hence decrease in the concentration of free drug could be due to conformational changes caused by the simultaneous binding of the two drugs.

Meloxicam: In the case of meloxicam, the presence of chlorpromazine caused a small decrease while ceftriaxone caused a significant decrease in the concentration of free drug. Our previous results /7/ have shown that meloxicam alone causes conformational changes in the HSA molecule. The presence of competing drugs (chlorpromazine/ceftriaxone) causes more unfolding, and therefore the binding of meloxicam increases in the presence of these drugs.

Gliclazide, on the other hand, had a negligible effect on the binding of meloxicam.

Nimesulide: Chlorpromazine and gliclazide caused a small increase in the concentration of free nimesulide. Results reported previously have shown that nimesulide causes conformational changes in the HSA molecule and it displaces both site I and site II probes. The small decrease in the binding of nimesulide in the presence of gliclazide and chlorpromazine showed that nimesulide and these drugs bind at different sites and the displacement is non-competitive. Ceftriaxone sodium had a negligible effect.

Etoricoxib: In the case of etoricoxib, gliclazide had a negligible effect while chlorpromazine and ceftriaxone resulted in a small decrease in the concentration of free drug. A small increase in the binding of etoricoxib in the presence of chlorpromazine and ceftriaxone sodium can again be attributed to conformational changes.

Chlorpromazine hydrochloride: Binding of chlorpromazine increased in the presence of celecoxib and meloxicam while it decreased in the presence of valdecoxib, parecoxib sodium, nimesulide and etoricoxib. Altered binding of this drug can be due to the fact that the structural changes caused by chlorpromazine in HSA /16/ are further enhanced in the presence of other drugs. The hydrophobicity of the competing drugs also seems to play a role since celecoxib and meloxicam are relatively more hydrophobic than the other COX-2 drugs studied.

Gliclazide: Amongst the various COX-2 inhibitors, the presence of valdecoxib and parecoxib sodium decreased the binding of gliclazide thereby increasing the percentage of free drug by about 20%. Other COX-2 inhibitors had practically no effect on the binding of gliclazide to HSA. Our results /8/ have shown that valdecoxib binds at site II whereas Fujii *et al.* /13/ showed that gliclazide binds to site I, but at a region distinct from the warfarin-binding region. However, it has been reported /17/ that gliclazide also possesses a secondary binding site. It appears that the secondary binding site of gliclazide overlaps with the primary binding site of valdecoxib, resulting in displacement of one drug in the presence of the other.

Ceftriaxone sodium: Valdecoxib and meloxicam decreased the binding of ceftriaxone sodium while other drugs had a negligible effect. The percentage of free ceftriaxone sodium increased by about 8% in the presence of valdecoxib and 12% in the presence of meloxicam. The small increase in the case of valdecoxib cannot be due to competitive displacement; small structural changes in the albumin molecule due to the presence of the two drugs may be responsible. It has been shown /7/ that meloxicam displaces both site I and site II probes and it can thus affect the binding of ligands at both sites. The binding of ceftriaxone sodium, a site I ligand, is therefore decreased in the presence of meloxicam.

CONCLUSIONS

The following conclusions can be drawn from the results presented above:

- In most cases, the presence of a competing drug caused structural changes in the HSA molecule, resulting in an increase or decrease in the concentration of free drug.
- The ability of ceftriaxone sodium, a large and polar molecule, to alter the binding of other drugs, and the ability of other drugs to alter the binding of ceftriaxone sodium, was either small or negligible.
- The percentage of free celecoxib increased by more than 30% in the presence of chlorpromazine. The percentage of free valdecoxib in the presence of gliclazide and gliclazide in the presence of valdecoxib increased by more than 20%. The percentage of free celecoxib in the presence of gliclazide, free chlorpromazine in the presence of celecoxib and meloxicam, free gliclazide in the presence of parecoxib sodium and free ceftriaxone sodium in the presence of meloxicam, increased by more than 10%. The percentage of free meloxicam in the presence of ceftriaxone sodium decreased by more than 10%. In other cases, either the effect was small (less than 10%) or there was practically no effect.

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