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The Effect of Co-administered Drugs on Oxaprozin Binding to Human Serum Albumin

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Abstract

The binding of the non-steroidal anti-inflammatory drug oxaprozin to human serum albumin was studied by bioaffinity high-performance liquid chromatography using a column based on immobilized human serum albumin.

Displacement studies using marker compounds for the major drug binding sites showed that oxaprozin has a high affinity for the benzodiazepine/indole site and binds to the warfarin site but with a significantly lower affinity.

Biochromatography and ultrafiltration techniques were used to screen for possible competition and allosteric interactions between oxaprozin and potential co-administered drugs including non-steroidal anti-inflammatory drugs, antipyretics, hypoglycaemics, inhibitors of angiotensin-converting enzyme, anaesthetics, metal ions and anticancer agents. Competition occurred mainly with drugs bound at the benzodiazepine site (benzodiazepines, various non-steroidal anti-inflammatories).

Oxaprozin is a non-steroidal anti-inflammatory drug (NSAID) active on the signs and symptoms of arthritis (Brown et al 1968; Todd & Brogden 1986). Oxaprozin is well tolerated and has fewer side-effects than aspirin (Rosenthale et al 1974). Its pharmacokinetics are characterized by a long half-life (50–60 h) which makes the drug suitable for once daily administration (Janssen et al 1980; Chiang et al 1982). It is highly bound to serum proteins (>99%) at therapeutic levels and most or all of this binding occurs on human serum albumin (HSA) (Chafetz & Philip 1982; Homon et al 1982; Chiang et al 1984).

Interferences between highly HSA-bound drugs can arise from their competition for the same site. The co-administration of two drugs which bind to the same site often leads to an increase in the free level of the drug with the lowest affinity with a potentiation of its pharmacological effect (McElnay & D'Arcy 1983). Alternatively, the binding of one drug can increase the affinity of the other for the protein by an allosteric interaction.

A prerequisite for the study of drug–drug protein binding interactions is the determination of the binding of the binding site. In this study, an in-vitro model based on HSA immobilized on high-performance liquid chromatographic stationary phases (HSA-SP) was used to identify oxaprozin primary binding sites on HSA and to screen for potential interferences with other drugs.

HSA-SP has proven to be a useful tool to probe drug–protein interactions (Domenici et al 1990, 1991; Aubry & McGann 1994). Studies have established that the immobilized HSA retained the properties of the native protein (Domenici et al 1990, 1991; Aubry & McGann 1994). The experimental model is based on displacement chromatography and follows the effect of the addition of a competitive compound to the mobile phase on the chromatographic retention of a drug of interest. All drug compounds can be used as ligands (injected onto the column) or as displacers (added to the mobile phase).

In the development of new drugs, the use of an HSA-SP can help anticipate the interactions of a drug with HSA and the related consequences for pharmacokinetics, efficacy and toxicity as well as to indicate possible incompatibilities with other agents (Aubry & McGann 1994). Previous studies have shown that this approach can readily identify drug–drug interactions including anti-cooperative (competitive displacement) and co-operative or non-co-operative allosteric interactions.

Since it was first developed as a chiral stationary phase, HSA-SP has been especially useful to investigate stereoselective binding to HSA (Domenici et al 1990, 1991; Aubry & McGann 1994). If drug binding to HSA is stereoselective, each stereoisomer will be eluted with a different retention time and its particular behaviour can be monitored separately.

Despite good correlation between the results obtained on HSA-SP and in-vivo observations, this approach has never been used in the development of a new therapeutic agent to assess drug–HSA and drug–drug interactions related to HSA binding. The objective of this study was to determine the effect on oxaprozin binding to HSA of the co-administration of a series of commonly used therapeutic agents. The affinity of oxaprozin for each of the two major drug binding sites on HSA, the warfarin site and the benzodiazepine site, was evaluated using displacement chromatography and marker compounds for each site. Screening for potential interaction was performed using oxaprozin as a ligand (injected on the column) or as a displacer (added to the mobile phase).

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Materials and Methods

Chemicals

The compounds used in this study were obtained from the following suppliers: oxaprozin from Searle (Skokie, IL); chlordiazepoxide, (*R,S*)-ketoprofen, piroxicam, D-tryptophan, L-tryptophan, aspirin, acetaminophen, chlorpropamide, glyburide, lithium carbonate, gold sodium thiomalate, phenylbutazone, oxazepam, mefenamic acid, captopril, lignocaine, bupivacaine and adrenaline from Sigma Chemical Co. (St Louis, MO, USA); (*R,S*)-ibuprofen, *R*-ibuprofen and *S*-ibuprofen from Upjohn Laboratories (Kalamazoo, MI, USA); (*R,S*)-warfarin, *R*-warfarin and *S*-warfarin from DuPont Merck Pharmaceutical Company (Wilmington, DE, USA); 2,3,5-tri-iodobenzoic acid from Dr D. Carter (NASA, Huntsville, AL); methotrexate and leucovorin from Lederle Laboratories (Pearl River, NY, USA); hydroxychloroquine from Sanofi-Winthrop (New York, NY, USA), enalaprilat from Merck Sharp and Dohme Research Laboratories (Rahway, NJ, USA); tamoxifen from Zeneca Pharmaceuticals (Macclesfield, Cheshire, UK). Other chemicals were of analytical grade and were obtained from local suppliers.

Chromatography

HPLC experiments were carried out isocratically using a modular HPLC system that consisted of a Spectroflow pump, a variable wavelength UV detector, a Data-Jet-integrator, all from Thermo Separation Products (San Jose, CA, USA) and a temperature controller (Fiatron System, Milwaukee, WI, USA). HSA columns (15 × 4·6 cm or 5 × 4·6 cm) were from Shandon HPLC (Runcorn, UK). The temperature was set at 37°C. The injection volume was 20 μL. The detector was set at a wavelength of 254 nm.

The mobile phase was composed of potassium phosphate buffer (0·1 M, pH 7·0): propan-1-ol, 94 : 6 (v/v). The flow rate was 0·8 mL min^{-1}. All mobile phases were filtered (0·45 μm filter) before use.

High-performance displacement chromatography

The chromatographic retention (expressed as a capacity factor k′) of a solute was used as an estimate of the degree of its binding to HSA. The percentage change in k′ was used as a measurement of the effect of displacers. The value of k′ was calculated from:

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where t_R is the retention time of the compound and t_0 is the elution time of an unretained compound. Each of the test solutes was injected and its capacity factor (k'_{init}) determined before the addition of the mobile phase additive. After addition of the mobile phase modifier and equilibration of the column, all compounds were chromatographed again and the new k′ (k'_{final}) calculated. The percentage change was then calculated as $(k'_{final} - k'_{init})/k'_{init} \times 100$. A negative figure indicated a decrease of retention and a positive figure, an increase. A change in k′ of 10% or more (absolute value) was considered to represent a meaningful interaction between the ligand and the displacer.

For chiral compounds, when the elution order was not known, we used the suffixes A and B to indicate the first and second eluted enantiomer, respectively. The elution order was determined by injecting the separate enantiomers if they were available.

Displacement curves

For the identification of the binding sites, oxaprozin was used as a displacer and its effect on the retention (measured as chromatographic capacity factors, k′) of marker compounds for binding at sites I or II was observed. Oxaprozin was added to the mobile phase at concentrations ranging from 0 to 20 μM. Markers for binding at the warfarin site included *R*-warfarin, *S*-warfarin and phenylbutazone. Markers for binding at the benzodiazepine site were *R*-oxazepam, *S*-oxazepam, chlordiazepoxide, D-tryptophan and L-tryptophan. Tri-iodobenzoic acid binds to both sites (He & Carter 1992).

The effect of increasing concentrations of oxaprozin was analysed using the equation developed previously (Noctor et al 1992a, b):

$$\frac{1}{(k' - X)} = \frac{V_m K_1 [A]}{K_2 m_L} + \frac{V_m}{K_2 m_L} \quad (2)$$

where K_1 and K_2 represent the affinity constants for the binding site at which the displacement occurs, of the displacer and the solute, respectively, V_m is the void volume of the system, m_L is the number of available moles of binding sites within the immobilized protein and [A] is the concentration of displacer. The term X is the contribution to the solute's k′ of binding to the sites that are not affected by the displacement and was obtained by iterative calculation. A linear relationship between $1/(k' - X)$ and displacer concentration indicates a simple competitive interaction at one site with binding at other binding sites. The term X equals zero when the displacement occurs at the unique binding site of the solute.

Ultrafiltration experiments

Ultrafiltration was carried out as previously described using a disposable Centrifree Micropartition System (Amicon, Beverly, MA) (Massolini et al 1993). Briefly, 50 μL 10 mM solution of the ligand was added to 1 mL of an HSA solution and incubated for 2 h at 37°C. For displacement studies, the test ligand and potential displacer were incubated with the protein for 2 h at 37°C. The solution was then transferred to the ultrafiltration unit and centrifuged for 15 min at 300 rev min^{-1}. After centrifugation, the concentration of ligand in the ultrafiltrate was determined by HPLC. The column used was a Lichrocart RP-18 10 × 4·6 cm (Merck). The mobile phase for tolbutamide consisted of phosphate buffer (pH 2·5, 100 mM)/acetonitrile 60 : 10 (v/v) and the retention times were 5·3 min for tolbutamide and 10 min for oxaprozin. The mobile phase for tamoxifen consisted of the same buffer with 50% (v/v) acetonitrile and the retention times were 5 min for oxaprozin and 13 min for tamoxifen. The mobile phase for warfarin consisted of the same buffer with 40% (v/v) acetonitrile and gave retention times of about 11·5 min for warfarin and 13 min for oxaprozin. The effluent corresponding to the elution peak of warfarin was collected, evaporated under nitrogen and re-injected onto a chiral chromatographic system for determination of the enantiomeric ratio. The column was a 150 × 4·6 mm i.d. Chiral

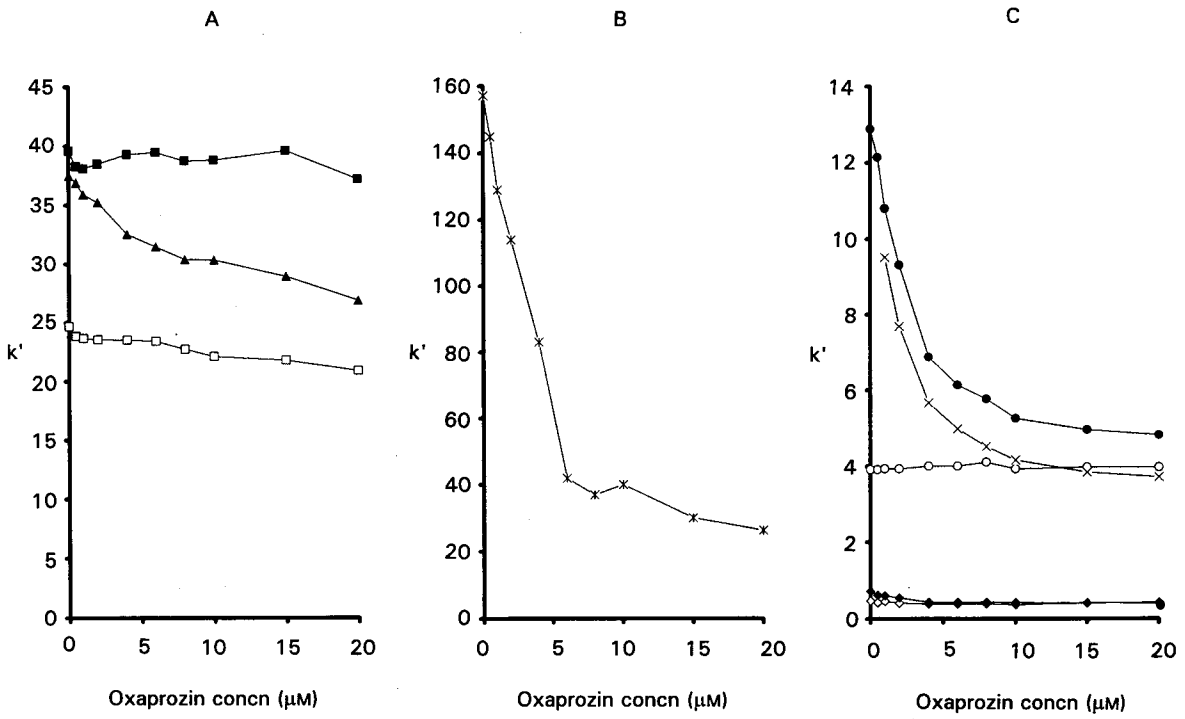

FIG. 1. Effect of increasing concentration of oxaprozin in the mobile phase on the capacity factor (k') on HSA-SP of A. site I markers, B. tri-iodobenzoic acid, C. site II markers. ■ *S*-warfarin; □ *R*-warfarin; ▲ phenylbutazone; ● *S*-oxazepam; ○ *R*-oxazepam; x chlordiazepoxide; ◆ L-tryptophan; ◇ D-tryptophan.

AGP (Regis), the mobile phase was composed of phosphate buffer (pH 5·5, 0·1 M), *n*-propanol 94 : 6 (v/v), the flow rate was 0·8 mL min^{-1} and the retention times of *S*- and *R*-warfarin were 22·2 and 28·9 min, respectively. Ultrafiltration experiments were carried out in duplicate.

Results and Discussion

Determination of oxaprozin binding sites

The exact number of drug binding sites on the HSA molecule has not been fully elucidated, but most workers agree on the existence of at least two major binding sites: the warfarin azapropazone, and the indole benzodiazepinone sites (also known as site I and site II, respectively) (Sudlow et al 1976; Sjöholm 1986; Feshke et al 1981; Wanwimolruk et al 1983). More recent studies using HSA-SP have demonstrated the existence of two distinct benzodiazepine sites: one non-saturable, low-affinity site which binds benzodiazepines in the P or M conformations and one saturable, high-affinity site which binds them in the M-conformation (Kaliszan et al 1992). This second site is believed to be equivalent to the previously described site II. Most achiral benzodiazepines and the *S*-enantiomer of the chiral benzodiazepines with a substituted oxygen atom on C3 were found to bind preferentially in the M-conformation. The respective *R*-enantiomers of the chiral benzodiazepines were bound in the P-conformation (Kaliszan et al 1992).

The effects of oxaprozin on markers for binding at site I and at site II are presented in Fig. 1. Site II markers *S*-oxazepam and chlordiazepoxide were significantly displaced by oxaprozin indicating that site II is a major binding site for oxaprozin. The fact that *R*-oxazepam was not affected by oxaprozin is consistent with displacement patterns previously reported for other NSAIDs, in particular ibuprofen. In a study of HSA binding of 21 benzodiazepines, it was observed that ibuprofen affected the binding of only eight compounds in the series (Noctor et al 1992b). Only the benzodiazepines which bound preferentially in the M-conformation (including the *S*-enantiomer of *O*-substituted compounds) were significantly displaced by ibuprofen. Here, a similar pattern is reported for oxaprozin, suggesting that, like ibuprofen, oxaprozin predominantly binds to the

Table 1. Parameters obtained from the plot of $1/(k' - X)$ against oxaprozin concentration.

Compound	Phenylbutazone	*S*-Oxazepam	Chlordiazepoxide
X	21·5	4·1	3·2
Intercept	0·0624	0·0910	0·0137
Slope	6201	68867	100630
r	0·9990	0·9906	0·09987
K_2 (M^{-1})	$9{\cdot}93 \times 10^4$	$7{\cdot}6 \times 10^5$	$7{\cdot}3 \times 10^6$

Table 2. Influence on the stereoselectivity of the HSA column of the addition of 10 μM oxaprozin to the mobile phase. $\alpha = k'2/k'1$ where k'2 and k'1 are respectively the second and the first eluted enantiomer of the compound.

Ligand	Stereoselectivity (α)	
	Without oxaprozin	With oxaprozin
Warfarin	1·60	1·77
Tryptophan	1·17	1·02
Oxazepam	3·20	1·21
Ibuprofen	*R*-Ibuprofen not eluted	1·00
Ketoprofen	1·25	1·32
Leucovorin	2·31	1·93

M-conformer benzodiazepine site. The mathematical model describing a simple competition at one site applied to both chlordiazepoxide and *S*-oxazepam. Table 1 summarizes the parameters of the curves for these two marker compounds. Two values were obtained for the affinity of oxaprozin for this site: $7{\cdot}6 \times 10^5\ M^{-1}$ (from the displacement of oxazepam) and $7{\cdot}3 \times 10^6\ M^{-1}$ (from the displacement of chlordiazepoxide).

The effect of oxaprozin on L- and D-tryptophan was also consistent with the above model. When used as a mobile phase additive in displacement chromatography, the effect of L-tryptophan was qualitatively similar to the effect of ibuprofen (it competed with the benzodiazepines which were mostly bound in the M-conformation) (Noctor et al 1992b). Oxaprozin displaces D- and L-tryptophan from the M-conformer benzodiazepine-binding site although because of the low retention of these two compounds, no calculation of affinity was possible.

Tri-iodobenzoic acid binds to both major sites on HSA (He & Carter 1992). The displacement curve of tri-iodobenzoic acid by oxaprozin showed two distinct segments suggesting a displacement from two sites (Fig. 1). The larger change in k' was due to the displacement from the high affinity (benzodiazepine) site, the second part of the curve was due to the displacement from the warfarin site (lower affinity site).

As shown in Fig. 1, oxaprozin also induced a significant displacement of site I markers phenylbutazone and *R*-warfarin. The more highly bound *S*-warfarin was not affected by oxaprozin. *R*-Warfarin showed smaller displacement (15% with the addition of 20 μM oxaprozin) than phenylbutazone (30% with the addition of 20 μM oxaprozin). The displacement of tri-iodobenzoic acid, *R*-warfarin and phenylbutazone indicate that site I is a minor binding site for oxaprozin. The displacement data for phenylbutazone fitted the mathematical model of competitive interaction at a single site. The affinity of oxaprozin for the warfarin site calculated from the displacement curves of phenylbutazone was $9{\cdot}93 \times 10^4\ M^{-1}$ (Table 1).

Previously published data suggested that oxaprozin

Table 3. Capacity factors of a series of ligands on HSA-SP in the absence and in the presence of 10 μM oxaprozin in the mobile phase.

	Mobile phase A k'	Mobile phase A+ oxaprozin (10 μM) k'	% change in k'
Compounds retained and affected (k' > 1)			
S-Ibuprofen	143	38	− 73
R-Ibuprofen	>200	38	< − 80
Ketoprofen A	52	26	− 50
Ketoprofen B	65	34	− 48
Piroxicam	38	28	− 26
Mefenamic acid	166	44	− 73
Chlorpropamide	5·8	4·5	− 25
Tolbutamide	11	8·2	− 27
Glyburide	108	88	− 18
R,6*S*-Leucovorin	2·5	1·9	− 33
Phenylbutazone	37	30	− 19
S-Oxazepam	13	5·3	− 62
Chlordiazepoxide	9·5	4·2	− 60
R-Warfarin	25	22	− 12
Compounds retained but unaffected			
Methotrexate	2·7	2·5	−9
(*R*,*S*)-Hydroxychloroquine	1·6	1·6	0
S-Warfarin	40	39	− 2·5
(*S*,6*S*)-Leucovorin	1·1	1	− 10
R-Oxazepam	3·9	4	1
Compounds not retained (k' < ou = 1)			
Adrenaline	0·3	0·3	
Aspirin	0·5	0·4	
Captopril	0·4	0·4	
Lignocaine	0·5	0·5	
Bupivacaine	0·8	0·8	
Paracetamol	0·5	0·1	
D-Tryptophan	0·5	0·4	
L-Tryptophan	0·7	0·4	

Table 4. Capacity factor of oxaprozin on HSA-SP in the absence and in the presence of drugs used as displacers.

	Mobile phase A Oxaprozin k′	Mobile phase A+ displacer Oxaprozin k′	% change in k′
Compounds reducing the binding of oxaprozin (| % change | > ou = 10)			
(*R*)-Warfarin (10 μM)	28·2	25·3	−10
Phenylbutazone (10 μM)	23·6	20	−15
R-Ibuprofen (10 μM)	15·4	13·5	−13
Mefenamic acid (10 μM)	13·6	9·6	−30
Chlordiazepoxide (30 μM)	26·1	13·3	−49
Aspirin (50 μM)	23·3	16	−31
Sodium aurothiomalate (50 μM)	21·3	17·9	−16
Lignocaine	32·1	28·6	−11
Enalapril (50 μM)	21·9	16·7	−24
(*S*,6*S*)-Leucovorin (50 μM)	34·4	27·1	−21
(*R*,6*S*)-Leucovorin (50 μM)	28	24	−14
Compound increasing the binding of oxaprozin (| % change | > 10)			
Captopril (50 μM)	29·8	36·3	22
Compounds which did not affect the binding of oxaprozin			
Adrenaline (50 μM)	32·2	30	−7
Tamoxifen (10 μM)	28·3	25·8	−9
S-Ibuprofen (10 μM)	14·7	14·5	−2
Chlordiazepoxide (10 μM)	15·1	14·4	−5
Bupivacaine (50 μM)	41·3	39·4	−4
Lithium carbonate (50 μM)	15·9	15·2	−5
Paracetamol (50 μM)	41·9	41·7	−0·5
Sodium aurothiomalate (10 μM)	21·6	20·4	−5·5
Tolbutamide	21	20·2	−4

binding to HSA was essentially non-specific (Chiang et al 1984). This study shows that oxaprozin significantly binds to both major drug binding sites on HSA. Further proof of the specificity of the binding at the benzodiazepine site is the finding that the addition of oxaprozin markedly decreases the enantioselectivity of the binding of both oxazepam and tryptophan (Table 2).

Drug interactions with oxaprozin for binding to HSA

The screening for interferences was carried out using oxaprozin first as a displacer, then as a ligand. The capacity factor of the ligands before and after addition of 10 μM oxaprozin as well as the percentage of change in k′ are presented in Table 3. Among the ligands tested, eight (aspirin, paracetamol, captopril, lignocaine, bupivacaine, adrenaline, D-tryptophan, L-tryptophan) had little retention on HSA-SP ($k' \leqslant 1$), suggesting that they have a relatively low binding to the protein. The eighteen remaining compounds were retained on HSA-SP ($k' > 1$). The capacity factor of oxaprozin before and after addition of different displacers and the percentage change in k′ are presented in Table 4. Interactions of warfarin, tolbutamide and tamoxifen with oxaprozin were also studied by ultrafiltration. Binding percentages of warfarin, tolbutamide and tamoxifen in the absence and in the presence of oxaprozin and binding percentages of oxaprozin in the absence and in the presence of the other three drugs are summarized in Table 5.

Interactions with warfarin. As mentioned above, oxaprozin did not affect the binding of *S*-warfarin and only slightly decreased the binding of *R*-warfarin. This stereoselective interaction resulted in an increase in the enantioselectivity α (Table 2). When used as a displacer, racemic warfarin induced a 10% change in the retention of oxaprozin (Table 4). As measured by ultrafiltration, no change was observed in the binding of *S*-warfarin in the presence of oxaprozin, but there was a 10% decrease in the binding of *R*-warfarin. Oxaprozin was also displaced by racemic warfarin reducing the bound fraction from 98·5 to 96%. The previous study which suggested that warfarin and oxaprozin did not displace each other from their binding sites (Chiang et al 1984) was partly contradicted by the present study.

Table 5. Binding percentages of warfarin, tolbutamide and tamoxifen in the absence and in the presence of oxaprozin and binding percentages of oxaprozin in the absence and in the presence of the other three drugs determined by ultrafiltration.

Displacers	Oxaprozin	Tamoxifen	Tolbutamide	*R*-Warfarin	*S*-Warfarin
None	98·5	99·6	96·5	93·0	94·5
Oxaprozin		99·7	95·5	83·1	91·7
Tamoxifen	96·4				
Tolbutamide	99·7				
Warfarin	96·0				

Oxaprozin selectively displaces the *R*-enantiomer of warfarin. It has been observed in-vivo that oxaprozin did not change the prothrombin time in patients stabilized on warfarin (Todd & Brogden 1986). However, no data on the plasma concentrations of the unbound drugs were given. The displacement of the *R*-enantiomer of warfarin only by oxaprozin is consistent with the observed absence of pharmacological effect on coagulation since *R*-warfarin is one-eighth as potent as the *S*-enantiomer and a decrease in protein binding of the *R*-enantiomer only is unlikely to be clinically relevant. No change in the dosage of the anticoagulant should be necessary if a patient is treated with oxaprozin.

Interactions with other NSAIDs. All NSAIDs were displaced by oxaprozin. Profens and mefenamic acid underwent a greater displacement than piroxicam or phenylbutazone. This can be explained by piroxicam and phenylbutazone being bound primarily to the warfarin site while profens and mefenamic acid are mainly bound to the benzodiazepine site (unpublished results). As shown in Table 2, the displacement of ibuprofen was accompanied by a loss of enantioselectivity indicating its displacement from a stereoselective site. On the other hand, the displacement of ketoprofen was accompanied by an increase in stereoselectivity indicating its displacement from a non-stereoselective site or from a site with a higher affinity for ketoprofen A than for ketoprofen B. When used as displacers at the same concentration (10 μM), *R*-ibuprofen was able to displace oxaprozin but the less bound *S*-ibuprofen was not. This suggests that oxaprozin has a higher affinity than *S*-ibuprofen for the benzodiazepine site.

Aspirin and paracetamol were not significantly retained on the HSA column and no conclusions could be made regarding the effect of oxaprozin. However, when used as a displacer, aspirin (50 μM) produced a 30% displacement of oxaprozin. Aspirin can engage in different types of interactions with HSA. As with many small acidic compounds, aspirin has an affinity for both site I and II (He & Carter 1992). It has been reported that it also selectively acetylates one amino acid (lysine 199) situated in the warfarin site (Hawkins et al 1968). The binding of aspirin to immobilized HSA was reversible and after washing the column regained its original chromatographic properties. Paracetamol at a concentration of 50 μM had no effect on the retention of oxaprozin. These results are consistent with previous studies reporting no major interaction between paracetamol and oxaprozin (Scavone et al 1986), but an interaction between aspirin and oxaprozin for binding to plasma proteins (Todd & Brogden 1986).

Interaction with hypoglycaemic drugs. The hypoglycaemic drugs chlorpropamide, tolbutamide and glyburide underwent little displacement by oxaprozin (13, 19 and 9%, respectively). Glyburide exhibited a much higher retention ($k' = 108$) than tolbutamide or chlorpropamide ($k' = 11$ and 5·8, respectively) indicating that it is more highly bound than its homologues. This is consistent with previous reports which had shown that the affinity constant of glyburide was at least one order of magnitude higher than the affinity of chlorpropamide or tolbutamide (Crooks & Brown 1974). The number or localization of binding sites for sulphonylureas remain unclear. A study of bovine serum albumin binding of a series of sulphonylureas by dialysis suggested that glyburide interacted with one class of binding site while chlorpropamide and tolbutamide interfered with two classes of binding site (Hsu et al 1974). Another study using fluorescent probes suggested that on HSA, glyburide was bound to a totally different site from tolbutamide and chlorpropamide. This study also suggested that chlorpropamide and tolbutamide bind to the warfarin site, but that binding of glyburide tended to increase the affinity of warfarin for the protein (Crooks & Brown 1974). Using HSA immobilized in microparticles and diazepam and warfarin as marker compounds, it was found that all three drugs significantly reduced the binding of marker drugs for both sites (Sjöholm et al 1979). The experiment described here cannot differentiate between a competitive interaction and an anti-co-operative, allosteric interaction. However, considering the difference of binding affinities between glyburide and chlorpropamide or tolbutamide, if all three molecules were bound to the same site, then tolbutamide and chlorpropamide would undergo a much larger displacement than the most highly bound glyburide. In our experiments, the displacement of all three sulphonylureas was of the same order of magnitude. The relatively small changes in the retention of tolbutamide and chlorpropamide are consistent with their being displaced from oxaprozin's minor binding site (warfarin site). The effect on glyburide could be due to a displacement from the same (warfarin) site while the main part of the binding occurred at a different site not affected by oxaprozin. When used as a displacer at a concentration of 50 μM, tolbutamide showed no significant effect on the retention of oxaprozin. Using ultrafiltration, a small displacement of tolbutamide (from 96·5 to 95·5% bound) by oxaprozin was observed while the effect on oxaprozin was a small increase in binding (from 98·5 to 99·7%). While both chromatography and ultrafiltration indicated a limited displacement of tolbutamide, the effect of tolbutamide on oxaprozin was in opposing senses. However, it must be noted that drug concentrations used in ultrafiltration were much higher than those used in chromatography and exceeded therapeutic levels for most drugs. Differences in concentration may explain these apparently conflicting results.

Interaction with tamoxifen. Tamoxifen is highly bound (99·7%) to plasma protein, mainly to HSA (Sipila et al 1988; Lien et al 1989). Binding occurs at a binding site specific to this compound (Sjöholm et al 1979). Tamoxifen could not be eluted from the HSA column under the conditions of the study and the effect of oxaprozin on its binding could not be determined. However, when added to the mobile phase, tamoxifen did not significantly affect oxaprozin binding. When the interference was studied by ultrafiltration, tamoxifen seemed to reduce the binding of oxaprozin but was not affected by the presence of oxaprozin. These results are consistent with the existence of a unique tamoxifen binding site.

Interaction with methotrexate. Fatal or near fatal interactions with methotrexate have been reported for a

number of NSAIDs including profens, salicylate, diclofenac and phenylbutazone (Thyss et al 1986). The principal cause of this toxicity is believed to be an inhibition of the renal secretion of methotrexate. Since methotrexate has a relatively low protein binding (50%) (Paxton 1981), its displacement from plasma protein is not expected to be of major significance. Methotrexate is displaced from HSA by aspirin (Thyss et al 1986). Here, oxaprozin induced only a negligible displacement (less than 10%) of methotrexate.

Interactions with hydroxychloroquine. Hydroxychloroquine has been reported to stereoselectively bind to plasma proteins, with the *S*-enantiomer being 64% bound and the *R*-enantiomer 37% (McLachlan et al 1993). Binding was stereoselective on both HSA and α-acid-glycoprotein but with reversed order of affinity (McLachlan et al 1993). *S*-Hydroxychloroquine was more highly bound to HSA than its antipode. The opposite was found on α-acid-glycoprotein. The enantiomers of hydroxychloroquine were not separated on the HSA column, although they were retained in the conditions of the study. The retention of hydroxychloroquine was not affected by the presence of oxaprozin in the mobile phase suggesting that hydroxychloroquine does not bind to either of oxaprozin's binding sites.

Interactions with leucovorin. Only the most retained diastereoisomer of leucovorin, (6*R*)-leucovorin, was notably affected by oxaprozin. As previously observed for chlorpropamide and tolbutamide, the displacement was relatively small considering that (6*R*)-leucovorin is not highly retained on HSA-SP. (6*S*)-Leucovorin underwent a 9% displacement but its capacity factor was only 1·1 on HSA-SP. When used as displacers, (*R*,6*S*)- and (*S*,6*S*)-leucovorin induced a 15 and 20% change, respectively, in the retention of oxaprozin. These relatively small changes in binding are compatible with a displacement from the low affinity site of oxaprozin—i.e. the warfarin site.

Interaction with anaesthetics. Anaesthetic drugs lignocaine and bupivacaine are 60–80 and 90% protein-bound in plasma, respectively (Wood 1986). However, their chemical properties and reported drug interactions suggest that binding is predominantly on α-glycoprotein and not on HSA (Wood 1986; Krauss et al 1986; Routledge 1986). Both drugs show little retention on the HSA column under the conditions of the study. When added to the mobile phase, bupivacaine did not interact with oxaprozin binding while lignocaine induced a relatively small but significant displacement of oxaprozin.

Interactions with angiotensin-converting enzyme (ACE) inhibitors. The ACE inhibitor, captopril, is 25–30% protein-bound in-vivo (Das et al 1989) and is not retained on the HSA column. Captopril has been shown to bind covalently to HSA through the formation of a disulphide bond (Park et al 1982; Keire et al 1993). NMR analysis showed that, in addition, captopril rapidly associates with HSA to form noncovalent binding (Keire et al 1993). Both types of binding interactions were reversible. Captopril displaces lactate from its binding sites on HSA, in-vitro (Keire et al 1993), and is displaced by glyburide and digoxin from egg albumin (Das et al 1989). An increase in the retention of oxaprozin on the HSA column was observed with the addition of captopril to the mobile phase, indicating a possible allosteric interaction between the two drugs. Despite the possibility of covalent binding, captopril did not cause an irreversible change of the HSA column.

Although it belongs to the same pharmacological class as captopril, enalaprilat has a very different chemical structure and does not engage in the same types of binding interactions. The extent of HSA binding of enalaprilat has not been reported. According to this study, enalaprilat did not have a high retention on the HSA column suggesting a relatively low HSA binding. When used as a displacer, enalaprilat (50 μM) decreased the retention of oxaprozin, although it was not clear from which site.

Interactions with gold and lithium ions. Gold has been reported to be about 95% protein-bound in plasma in-vivo, mostly to albumin (Danpure et al 1979). The site of interaction on albumin is the thiol group of cystein 34. In this study, metal ions did not affect the binding of oxaprozin. A 16% decrease in retention was observed with gold at a concentration of 50 μM; however, this effect seemed to be due to an irreversible change in the protein at this concentration since the performance of the column could not be regained, even after repeated washing of the column.

These results indicate that oxaprozin interacts mainly with compounds bound at the benzodiazepine site (benzodiazepines, most NSAIDs) by increasing their free concentration. As the warfarin site is a minor site for oxaprozin, there will also be some interaction with compounds bound to this site, e.g. warfarin, phenylbutazone, piroxicam, leucovorin, enalaprilat and probably hypoglycaemic drugs. Metal ions and basic drugs such as anaesthetics and captopril did not affect the binding of oxaprozin, nor did drugs bound to sites other than site I and II, such as hydroxychloroquine and tamoxifen. Results of ultrafiltration experiments were in good agreement with chromatographic results.

Biochromatography provided a rapid and easy screening test for potential drug interactions on HSA. When in-vivo data were available, as for warfarin, biochromatographic results were able to explain at a molecular level the observed pharmacological effect. The use of immobilized HSA can rapidly identify drug interactions which should be included in future studies in-vivo.

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