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COMMUNICATIONS

The binding of flurbiprofen to plasma proteins

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The binding of flurbiprofen to human serum albumin and normal plasma was measured using ultracentrifugation. Flurbiprofen was bound in excess of 99% in all cases. Over the concentration range normally associated with therapy the data could be adequately described by a one-site binding model. The association constant decreased with increasing protein concentration, being $3{\cdot}04 \times 10^6\ M^{-1}$ at an albumin concentration of 2 g/100 ml and $1{\cdot}19 \times 10^6\ M^{-1}$ at a concentration of 4 g/100 ml. Flurbiprofen binding showed no dependence on pH over the range 7·0 to 8·0.

Flurbiprofen (2-(2-fluoro-4-biphenyl)propionic acid pK_a 4·2) is one of the newer propionic acid group of non-steroidal anti-inflammatory drugs (NSAID) used for the treatment of inflammatory disorders. In man the drug has a relatively short half-life (3·5–4·0 h) and a small volume of distribution (<10 litres) (Cardoe et al 1977). The latter is consistent with its tight binding to plasma proteins, particularly albumin. In human serum it is bound in excess of 99% at therapeutic concentrations with an association constant of $4{\cdot}1 \times 10^6\ M^{-1}$ (Kober & Sjöholm 1980).

The impetus for the present work comes from clinical studies of the distribution of flurbiprofen into synovial fluid of rheumatoid arthritic patients where pH and albumin concentration differ from the corresponding values in plasma and are variable (Wallis & Simkin 1983). Therefore we have investigated the binding of flurbiprofen to purified human serum albumin and plasma obtained from normal volunteers. In particular we examined the pH and protein concentration dependence of flurbiprofen binding.

Materials and methods

Protein binding was measured using ultracentrifugation as described previously (Aarons et al 1983). In separate experiments it was determined that flurbiprofen did not bind to the polycarbonate tubes used in the study. Human serum albumin (HSA) (gift from Kabi AB—used as received) was made up in 0·05 M, pH 7·4 phosphate buffer. Pooled plasma was obtained from normal volunteers who were not receiving drugs at the time blood was taken. Flurbiprofen and [^{14}C]-labelled flurbiprofen (specific activity 22 μCi mg^{-1}) were supplied by the Boots company. All other chemicals were of analytical reagent grade.

The binding of flurbiprofen to HSA—2 g/100 ml and 4 g/100 ml—and plasma was measured over the concentration range 4 to 100 mg $litre^{-1}$. The pH sensitivity of flurbiprofen binding was determined in a 2 g/100 ml HSA solution at flurbiprofen concentrations of 4 and 24 mg $litre^{-1}$ over a pH range of 7·0 to 8·0. pH did not vary by more than 0·1 during the centrifuge run. The dependence of flurbiprofen binding on protein concentration was determined at flurbiprofen concentrations of 24 and 100 mg $litre^{-1}$ and HSA concentrations between 0·5 and 8·0 g/100 ml.

Results

Binding to HSA and plasma. The coefficient of variation of the fraction of flurbiprofen unbound at concentrations of 4 and 24 mg $litre^{-1}$ (HSA concentration 4 g/100 ml) was 9·7 and 12%, respectively (n = 10). These relatively high values are due to the fact that the fraction unbound is approximately 0·15% and consequently the unbound flurbiprofen concentration is very small. The fraction unbound at 4 mg $litre^{-1}$, 0·13 ± 0·01% was significantly less ($P < 0{\cdot}05$) than at 24 mg $litre^{-1}$, 0·15 ± 0·02%. However, in the therapeutic range a difference of this magnitude would be of little consequence.

A Scatchard plot of the binding data obtained with 4 g/100 ml HSA is shown in Fig. 1. The estimated values of n, the number of bindings sites on the protein molecule, and K, the association constant, were 1·40 and $1{\cdot}19 \times 10^6\ M^{-1}$, respectively.

The corresponding figures for the data obtained with 2 g/100 ml HSA (Fig. 1) were 1·46 and $3{\cdot}04 \times 10^6\ M^{-1}$. Fig. 1 also shows the Scatchard plot obtained with plasma. The binding parameters were 1·10 and $6{\cdot}54 \times 10^5\ M^{-1}$.

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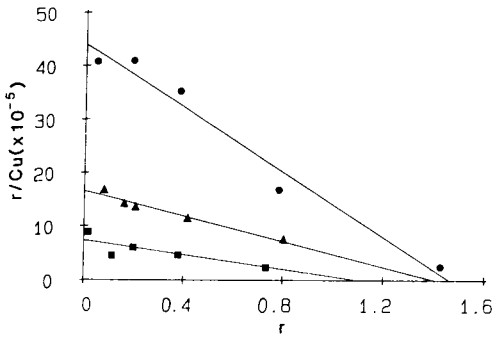

FIG. 1. Scatchard plots of the binding of flurbiprofen to 2 g/100 ml HSA (●); 4 g/100 ml HSA (▲); normal human plasma (■). r is the concentration of bound drug divided by protein concentration and Cu is the unbound drug concentration. Each point is the mean of two observations.

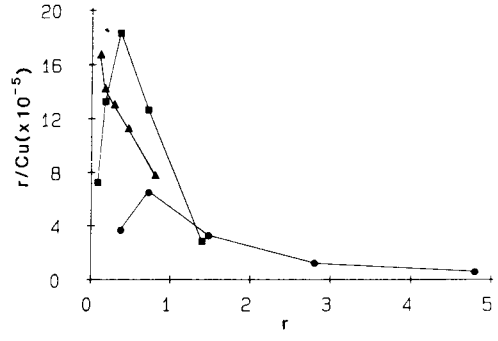

FIG. 2. Dependence of flurbiprofen binding on protein concentration. Flurbiprofen concentrations are 24 mg litre^{-1} (■); 100 mg litre^{-1} (●). (▲) are data obtained at a HSA concentration of 4 g/100 ml and varying drug concentration.

These values are in broad agreement with those obtained by Kober & Sjöholm (1980)—n = 1·4, K = 4·1 × 10^6 M^{-1} and those of Marchant (private communication), n = 1·1, K = 5·3 × 10^5 M^{-1}—given that these figures were obtained from experiments in serum rather than plasma.

pH sensitivity. The binding of flurbiprofen, 4 mg litre^{-1}, to HSA (2 g/100 ml) was not influenced by pH in the range 7·0 to 8·0.

Dependence on protein concentration. Scatchard plots for the dependence of binding on protein concentration are shown in Fig. 2. It is obvious that the plots—flurbiprofen concentrations of 24 and 100 mg litre^{-1}—are not superimposable, which they would be if the binding was independent of protein concentration. The data obtained at varying drug concentrations and a HSA concentration of 4 g/100 ml are plotted in Fig. 2 for comparative purposes. Since the data do not fit the model of a one-site binding isotherm it is not possible to estimate the parameters n and K from this data.

Discussion

Our results are in broad agreement with those of other workers and indicate that the binding of flurbiprofen to plasma proteins can be adequately described by a one-site binding model over the concentration range associated with therapy (<20 mg litre^{-1} Cardoe et al 1977). It is unlikely that changes in binding seen over this concentration range would manifest themselves in terms of non-linear pharmacokinetics.

The results for protein concentration dependence are less clear cut. Dependence of binding parameters on protein concentration has been noted previously for a variety of compounds (Bowmer & Lindup 1978). Co-operativity and protein–protein interactions are two explanations that have been put forward but no unambiguous experimental evidence exists to substantiate either.

The major problem associated with protein concentration dependence of binding is the difficulty in extrapolating from one protein concentration to another. The equation for a one-site binding model is

$$fu = [-D + \sqrt{D^2 + 4KC}]/2KC \qquad (1)$$

with D = 1 + nKP − KC where fu is the fraction of flurbiprofen not bound to protein, P is the molar protein concentration and C is the total (bound + unbound) molar flurbiprofen concentration. In the limit when K becomes large and C small, equation (1) tends to fu = 1/(nKP) and so if P decreases by a factor of 2, fu should increase by a factor of 2. In the case of flurbiprofen this is not observed. At a flurbiprofen concentration of 24 mg litre^{-1} the fraction unbound at a HSA concentration of 4 g/100 ml was 0·15% whereas at a HSA concentration of 2 g/100 ml it was 0·18%. This result is in keeping with the observation of Wanwimolruk et al (1983) that in rheumatoid arthritis patients the fraction of flurbiprofen not bound in synovial fluid was the same as in plasma even though the albumin concentration in synovial fluid is only about one half of that of plasma. These results can be explained by a decrease in the association constant, as the protein concentration increases. At a HSA concentration of 2 g/100 ml the estimated association constant was 3·04 × 10^6 M^{-1} whereas at a HSA concentration of 4 g/100 ml it was only 1·19 × 10^6 M^{-1}. However, as seen from Fig. 2, the relation between binding, protein concentration and drug concentration is complex and therefore it is difficult to predict drug binding at one protein concentration based on results obtained at a different concentration.

In our studies, flurbiprofen binding was not influenced by pH (over the range 7·0 to 8·0) and so the major determinant of the variability in flurbiprofen binding in synovial fluid is likely to be albumin

concentration. Variability in synovial fluid concentration is also determined by distribution into and out of the synovial cavity, which is thought to be diffusion limited and altered in synovitus (Wallis & Simkin 1983) and by variability in the corresponding plasma concentration. It is yet to be determined to what extent variability in binding contributes to the overall variability seen in synovial fluid concentration.

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The effect of xanthine derivatives on red blood cells: microelectrophoretic studies

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The effect of xanthine derivatives on the variation of surface charges of red blood cells has been investigated. Results of mobility curves showed that the derivatives increase the surface charges on the cells, whereas there is little effect on the surface charges of liposomes.

Pentoxifylline (xanthine derivative) can improve red cell (RBC) deformability; decrease blood viscosity, platelet aggregation and serum fibrinogen level; increase fibrinolytic activity and cerebral microcirculation and reduce peripheral vascular resistance (Grigoleit et al 1976; Volker 1976; Angelkort et al 1979; Marcel 1979; Takamatsu et al 1979). It was found to be useful in occlusive cerebral and peripheral vascular diseases (Gorbatschova et al 1978; Itoh & Satoh 1979; Satewachin et al 1978).

The mechanism of pentoxifylline in reducing the rigidity of red cell membranes to improve deformability due to the elevation of ATP concentrations, has been reported by Nakao (1974) and Stefanovich (1978). However, little work has been published on the effect of pentoxifylline on the surface charges of RBCs, as the magnitude of the surface charges on the cells can influence their aggregation. Therefore, we have used microelectrophoresis to investigate the variation of the surface charges of RBCs in the presence of the xanthine derivatives: pentoxifylline, aminophylline, xanthinol niacinate, caffeine and theophylline.

Materials and methods

Materials. Pentoxifylline (Hoechst, ROC), aminophylline (Sigma, USA), xanthinol niacinate (Italchimici, Italy), caffeine (Sigma, USA), theophylline (Delta Synthetic Co., ROC), cholesterol (Sigma, USA) and dicetyl phosphate (P.L. Chemicals Inc., USA) were used as received. Phosphatidylcholine was purified from fresh egg yolk by a two-step column procedure i.e. an alumina column and a silicic acid column (Singleton et al 1965).

Methods. A chloroform solution of phosphatidylcholine cholesterol and dicetyl phosphate at a molar ratio of 1·60 : 1·00 : 0·15 was prepared in a 500 ml round bottle flask and evaporated under reduced pressure at 37 °C to form a thin film on the flask. Phosphate buffer (0·067 M, pH 7·4) was added to the flask to give a concentration of lipid 1 mg ml^{-1}. Multilamellar liposomes were formed by constant vortexing for 5 min. The liposome dispersion was hydrated at 37 °C for 2 h.

Stock solutions of xanthine derivatives made in the phosphate buffer were added to 0·1 ml of fresh blood obtained from normal adult donors or 1 ml of liposome dispersion. The volume was made up to 20 ml with phosphate buffer to give the required concentration of the xanthine derivatives. The dispersion was incubated for 30 min at 25 °C before the measurement. The total volume (20 ml) of the dispersion was used for each measurement.

Mobility determinations on the RBCs and liposomes were carried out at 25 °C using a Rank MK II Microelectrophoresis Apparatus (Rank UK). The flat cell assembly and platinum electrodes were used. Ten individual particles were timed in both directions of the electric current to minimize the polarization of electrodes. The mobility is expressed by U = V/E where V is

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