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On the binding of cinmetacin and indomethacin to human serum albumin

M. T. MONTERO, R. POUPLANA, O. VALLS, S. GARCÍA, *Departament de Fisico-Química Biològica, Facultat de Farmàcia, Universitat de Barcelona, 08028-Barcelona, Spain*

The binding of two non-steroidal anti-inflammatory drugs, indomethacin and cinmetacin, to human serum albumin was studied by dynamic dialysis at 37 °C and pH 7·4. Cinmetacin is bound more than indomethacin. The affinity constant for the primary binding site is $4{\cdot}28 \times 10^6$ M^{-1} for cinmetacin and $1{\cdot}4 \times 10^5$ M^{-1} for indomethacin. The protein binding of indomethacin is decreased in the presence of cinmetacin.

Various techniques have been employed to study drug-protein interactions (Meyer & Guttman 1968a; Kurz et al 1977; Rowland 1980). We have used dynamic dialysis as a method to quantify the protein-binding of the two non-steroidal anti-inflammatory drugs (NSAIDs), indomethacin and cinmetacin. Both agents are arylacetic derivatives but cinmetacin has the more potent anti-inflammatory activity and lower toxicity than indomethacin (Komatsu et al 1973).

The quenching of the intrinsic fluorescence of human serum albumin (HSA) upon the binding of the two NSAIDs has been used to study drug-protein interactions and to calculate the binding parameters.

Materials and methods

Human serum albumin (essentially fatty acid-free) was purchased from the Sigma Chemical Company, St Louis. Drug samples were kindly donated by pharmaceutical companies and were used as received.

Drugs and albumin solutions were made up in phosphate buffer (pH 7·4, 0·066 M). The NSAIDs were dissolved in a few drops of 0·1 M NaOH and made up to the volume required with buffer, the pH being adjusted to 7·4. The total concentration of NSAIDs was 0·5 to 3×10^{-5} M and that of HSA $1{\cdot}0 \times 10^{-5}$ M.

A dynamic dialysis method, similar to that described by Meyer & Guttman (1968b), was used to study the binding of the NSAIDs to HSA. It was performed using Visking dialysis strips with a molecular weight cut off ≯12 000. The membranes were prepared by the method described by Briggs et al (1983). The apparatus used was similar to that described by Judis (1977). The protein-containing buffer solution was placed inside the dialysis sac which was immersed in buffer containing cinmetacin, indomethacin or a mixture of both drugs. Samples were taken from each side of the membrane at 2 h intervals until equilibrium had been reached. Dialysis was at 37 °C.

Samples from inside the sac containing the protein solution were measured in a Perkin-Elmer 204 fluorescence spectrophotometer and the fluorescence intensities were recorded directly from fluorimeter readings. The fluorescence intensity of HSA measured at a λ excitation 285 nm and λ emission 335 nm decreased proportionally with the fraction of drug bound to the protein. The initial fluorescence values of protein-containing buffer solution were considered to be 100% of fluorescence intensity. From these values the relative fluorescence intensity (RFI) of each fluorimeter reading was calculated as a percentage of initial fluorescence.

A dialysis run in the absence of protein showed that the diffusion was a first-order process with respect to the drug and the adsorption by the dialysis sac was negligible (Meyer & Guttman 1970).

In a phosphate buffer solution (pH 7·4, 0·066 M) the intrinsic fluorescence of indomethacin is low in the range of concentrations used and was not altered in the presence of HSA. Cinmetacin does not fluoresce under these conditions.

The polynomial equation that fitted the binding kinetics was determined to calculate the equilibrium time. Experimental fluorometric data were treated as described by Bordeaux-Pontier et al (1978).

Results

The curvilinear plots of the binding kinetics are displayed in Fig. 1. The relative fluorescent intensities of the two NSAIDs and that corresponding to a mixture of both NSAIDs in the presence of the same amount of HSA were plotted against time (h). For a total concentration of the mixture of $2{\cdot}11 \times 10^{-6}$ M ($1{\cdot}05 \times 10^{-6}$ M of cinmetacin + $1{\cdot}05 \times 10^{-6}$ M of indomethacin), the diffusion rate was less than the corresponding value for indomethacin ($2{\cdot}11 \times 10^{-6}$ M) but greater than the cinmetacin rate ($2{\cdot}11 \times 10^{-6}$ M). When the total concentration of the mixture ($4{\cdot}22 \times 10^{-6}$ M) matched the addition of individual concentrations, the diffusion rate was less than the sum for both drugs separately, although the difference with respect to the cinmetacin diffusion rate was very small. During the first 2 h, cinmetacin and the mixture caused a significant decrease in protein fluorescence. In all cases the diffusion rate values were in agreement with the calculated equilibrium times.

Table 1. Theoretical equilibrium times of indomethacin, cinmetacin and the mixture of both drugs using dynamic equilibrium dialysis in the presence of the same amount of HSA.

Indomethacin										
Time (h)	—	18·5	19·1	18·3	16·1	12·5	—	13·9	—	—
Concn (10^{-6} M)	—	1·46	2·11	2·92	3·92	4·62	—	8·36	—	—
Cinmetacin										
Time (h)	20·7	18·3	15·7	—	14·7	11·3	—	9·10	—	—
Concn (10^{-6} M)	0·75	1·40	2·10	—	3·99	4·62	—	8·36	—	—
Mixture of both drugs										
Time (h)	—	—	19·3	—	13·9	—	11·3	10·5	9·10	9·0
Concn (10^{-6} M)	—	—	2·11	—	3·98	—	5·86	8·34	9·24	16·7

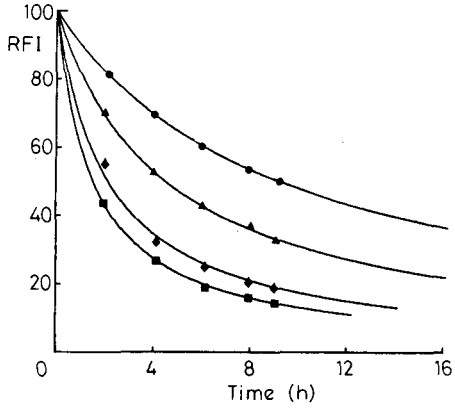

FIG. 1. Plots of relative fluorescence intensities (RFI) as a function of time (h) for indomethacin (●), cinmetacin (◆) and a mixture of both drugs (▲: at the same total concentration, ■: at double total concentration).

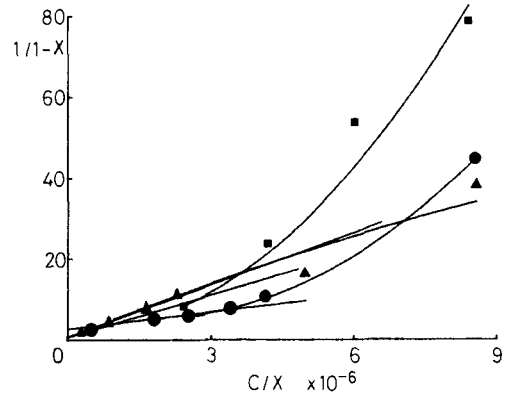

FIG. 3. Scatchard plots of the binding to HSA of indomethacin (●), cinmetacin (▲) and the mixture of both drugs (■) in the presence of the same amount of HSA.

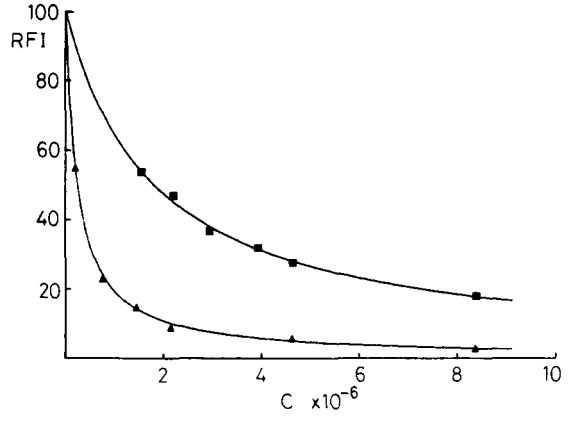

FIG. 2. Relative fluorescence intensities of HSA against the total concentration of indomethacin (■) and cinmetacin (▲).

The equilibrium times thus calculated for indomethacin, cinmetacin and the mixture of both drugs are reported in Table 1. For any given concentration, the cinmetacin equilibrium times were less than those for indomethacin. The differences between equilibrium times increase at high concentrations (e.g. 4 h for 8·36 × 10^{-6} M and 0·2 h for 1·40 × 10^{-6} M). The differences in the values obtained from the same concentration between the two NSAIDs could be observed as a characteristic of their binding to the HSA molecule. When the differences between the equilibrium time of the mixture are compared with the values obtained for each drug separately, the values of the mixture lie nearer to cinmetacin than to indomethacin values. For a total concentration of the mixture greater than 8·5–9·0 × 10^{-6} M the equilibrium times became constant.

Fig. 2 shows the variation in relative fluorescence intensity at the equilibrium conditions as a function of the total amount of drug dialysed. For a given concentration the increments became almost constant. There was a minimum value of 4·57 for cinmetacin and 13·03 for indomethacin for concentrations higher than 2·0 × 10^{-6} M. From Fig. 2 it can be concluded that the drug-protein binding was total at the calculated equilibrium time.

The binding of indomethacin to HSA was also studied at higher protein concentrations. The agreement between the results arising from the different protein concentrations may indicate that the binding is independent of the HSA concentration in the interval investigated (Hultmark et al 1975).

Table 2. Association constants (Ka) and number of primary binding sites (n_1) for indomethacin, cinmetacin and the mix of both drugs in the binding to HSA.

	Ka (10^6 M^{-1})	n_1
Indomethacin	1·4	2·71
Cinmetacin	4·28	0·87
Mixture of both drugs	3·5	0·69

Scatchard plots in Fig. 3 show a marked initial slope which allows the calculation of the binding parameters (Table 2), while at concentrations higher than $4{\cdot}0 \times 10^{-6}$ M results are less significant. These results can be explained by the presence of a high number of ligands with non-specific binding properties. The number of equivalent binding sites for both NSAIDs separately was two, while a value of four was found for the mixture. This fact could be explained as a non-competitive inhibition binding to the HSA molecule. The results concerning the association constant values for cinmetacin were higher than those for indomethacin. Results for the mixture were closer to those for cinmetacin than to indomethacin.

Dynamic dialysis indicates that cinmetacin binds to approximately one binding site of HSA with a high affinity constant and that indomethacin binds to the protein with approximately two binding sites but with a lower affinity constant. The fluorescence quenching of the protein is due to the energy transfer between drug and protein tryptophan and which occurs at only one of these sites for indomethacin. Therefore, cinmetacin and indomethacin must be bound in the region of the tryptophan residue and a competitive interaction for the same binding site on the protein has to take place.

When both drugs are bound to the protein, the resulting association of each can be influenced by the presence of the other. For the mixture of both drugs the calculated number of binding sites corresponds to a higher affinity constant for cinmetacin than for indomethacin.

REFERENCES

Bordeaux-Pontier, M., Chauvet-Deroudihle, M., Sarrazin, M., Briand, C. (1978) J. Chim. Phys. 75: 973–977

Briggs, C. J., Hubbard, J. W., Savage, C., Smith, D. (1983) J. Pharm. Sci. 72: 918–921

Hultmark, D., Borg, K. O., Elofsson, R., Palmer, L. (1975) Acta Pharm. Suec. 12: 259–276

Judis, J. (1977) J. Pharm. Sci. 66: 802–806

Komatsu, T., Saito, Ch., Awata, H., Sakai, Y., Inukai, T., Kurokawa, H., Yamamoto, H. (1973) Arzneimittel-Forsch./Drug Res. 23: 1690–1696

Kurz, H., Trunk, H., Weitz, B. (1977) Ibid. 27: 1373–1380

Meyer, M. C., Guttman, D. E. (1968a) J. Pharm. Sci. 57: 895–918

Meyer, M. C., Guttman, D. E. (1968b) Ibid. 57: 1627–1629

Meyer, M. C., Guttman, D. E. (1970) Ibid. 59: 33–38

Rowland, M. (1980) Ther. Drug Monit. 2: 29–37