Biochemical Pharmacology, Vol. 27, pp. 139 – 144. Pergamon Press, 1978. Printed in Great Britain

REPRESENTATION AND INTERPRETATION OF DRUG DISPLACEMENT INTERACTIONS

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(Received 27 June 1977; accepted 5 October 1977)

Several reports of clinically significant drug interactions, which have been attributed in part to drug displacement, appear in the literature - a noted example is the phenylbutazone-warfarin interaction [1,2,3]. The *in vitro* results of such drug displacement interactions are usually presented in terms of one of the linearisation procedures, such as the Scatchard plot [4] or the double reciprocal plot [5], commonly employed to represent single drug-protein binding data. The advantage of these linearisation procedures is that they give a convenient representation of the data and, when linear, aid in interpretation. In this note the limitations of one such linearisation, the Scatchard plot, are discussed and an alternative approach is suggested.

Consider the case of two drugs, A and B, that compete for a single binding site. Drug A is selected as the reference drug, while drug B is selected as the displacing drug. The equations describing the binding are the following:

$$C_T^A = C_u^A + \frac{nk_A C_u^A P_T}{1+k_A C_u^A + k_B C_u^B} \qquad (1a)$$

$$C_T^B = C_u^B + \frac{nk_B C_u^B P_T}{1+k_A C_u^A + k_B C_u^B} \qquad (1b)$$

where

$C_T^X \equiv$ total concentration of drug (X = A or B)

$C_u^X \equiv$ unbound concentration of drug (X = A or B)

$P_T \equiv$ total protein concentration

$n \equiv$ number of binding sites

$k_X \equiv$ equilibrium constant for drug (X = A or B)

Solving equation (1b) for C_u^B yields

$$C_u^B = \frac{-(1+k_A C_u^A + nk_B P_T - k_B C_T^B) + \sqrt{(1+k_A C_u^A + nk_B P_T - k_B C_T^B)^2 + 4k_B C_T^B (1+k_A C_u^A)}}{2k_B}$$

$$\equiv f(C_u^A, C_T^B) \qquad (2)$$

Substituting this result in (1a) and transforming to the Scatchard form gives

$$k_B P_T (r/c)^2 + k_A k_B P_T (r/c) r + (1 - n k_B P_T + k_B C_T^B) k_A (r/c) + k_A^2 r - k_A^2 n = 0 \qquad (3)$$

where $r/c = (C_T^A - C_u^A)/C_u^A P_T$

$r = (C_T^A - C_u^A)/P_T$

are the Scatchard axes. Equation (3) is the locus of a hyperbola which is concave to the r/c–r axes and has the following intercepts

$$\lim_{(r/c)\to 0} r = n \qquad \lim_{r \to 0} (r/c) = \frac{nk_A}{1+k_B f(0, C_T^B)}$$

where $f(0, C_T^B)$ is the unbound concentration of drug B in the absence of drug A.

Commonly it is implicitly assumed that in an *in vitro* displacement study the unbound concentration of the displacing drug is constant (and equal to

$f(0,C_T^B))$, in which case, as seen upon appropriately rearranging equation (1a) a straight Scatchard plot is obtained with slope $-k_A/(1+k_B f(0,C_T^B))$. The intercepts, $nk_A/(1+k_B f(0,C_T^B))$ and n are the same as equation (3). However, only the total concentration of the displacing drug (C_T^B) remains constant: drug B both displaces and is displaced by drug A. Equation (3) is plotted out in Scatchard form (Figure 1; solid lines) in the presence of varying total concentrations of drug B. The marked departure of these lines from the linear plots (dotted lines) obtained when the unbound concentration of drug B is assumed to be constant, and equal to $f(0,C_T^B)$, indicates the difficulty in interpreting Scatchard plots for drug displacement interactions.

In practice the situation is more complicated than depicted in the example above, in that both competitive and non-competitive interactions, as well as the possibility of multiple binding sites must be catered for. We have examined a more general approach to representing drug displacement interactions, based on Klotz's stoichiometric formulation[6]. Consider the following equilibria between protein P, drug A and drug B

$$A + P \overset{K_1}{\rightleftharpoons} AP \qquad B + BP \overset{K_4}{\rightleftharpoons} B_2P$$

$$B + P \overset{K_2}{\rightleftharpoons} BP \qquad B + AP \overset{K_5}{\rightleftharpoons} BAP$$

$$A + AP \overset{K_3}{\rightleftharpoons} A_2P \qquad A + BP \overset{K_6}{\rightleftharpoons} ABP$$

etc.

where K_i is the i^{th} equilibrium constant. The resulting binding isotherm for drug A is

$$C_T^A = C_u^A + \frac{(K_1C_u^A + 2K_1K_3(C_u^A)^2 + K_1K_5C_u^AC_u^B + K_2K_6C_u^AC_u^B + \dots)P_T}{(1+K_1C_u^A + K_1K_3(C_u^A)^2 + K_2C_u^B + K_2K_4(C_u^B)^2 + K_1K_5C_u^AC_u^B + K_2K_6C_u^AC_u^B + \dots)} \qquad (4)$$

with a corresponding equation for drug B. In order to fit equation (4) to the data one must measure both the total and unbound concentrations of the two drugs, as well as the total protein concentration.

In a preliminary investigation, we have used equilibrium dialysis to study the simultaneous binding of warfarin and salicylate to bovine serum albumin. Scatchard plots of the warfarin data for three differing total amounts of added

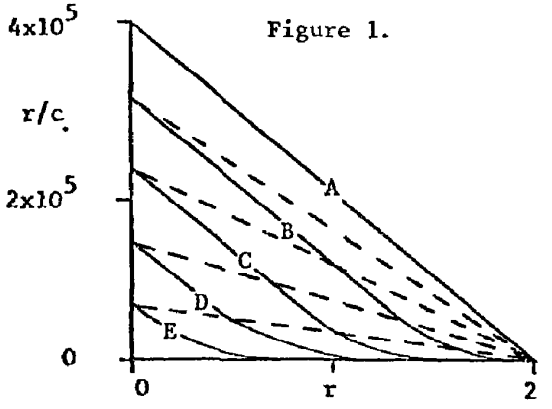

Figure 1.

Scatchard plots for competitive binding.

The appropriate Scatchard parameters used were:-

$n_A = n_B = 2$

$k_A = k_B = 2 \times 10^5 M^{-1}$

protein concentration = 5.8×10^{-4}M

A. Drug A alone
B. Drug A + 2.5×10^{-4}M drug B
C. Drug A + 5.0×10^{-4}M drug B
D. Drug A + 7.5×10^{-4}M drug B
E. Drug A + 1.0×10^{-3}M drug B

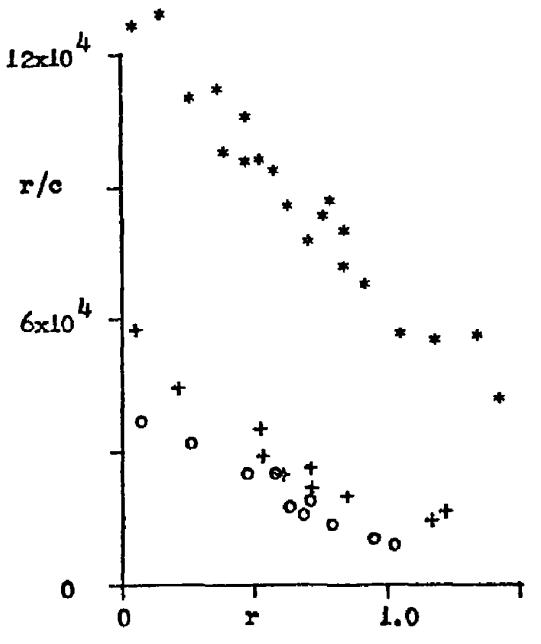

Figure 2. Scatchard plots for warfarin/salicylate interaction.

* Warfarin alone;

+ Warfarin + 2.191×10^{-3}M salicylate

o Warfarin + 3.146×10^{-3}M salicylate.

The total protein concentration was held constant at approximately 5.4×10^{-4}M.

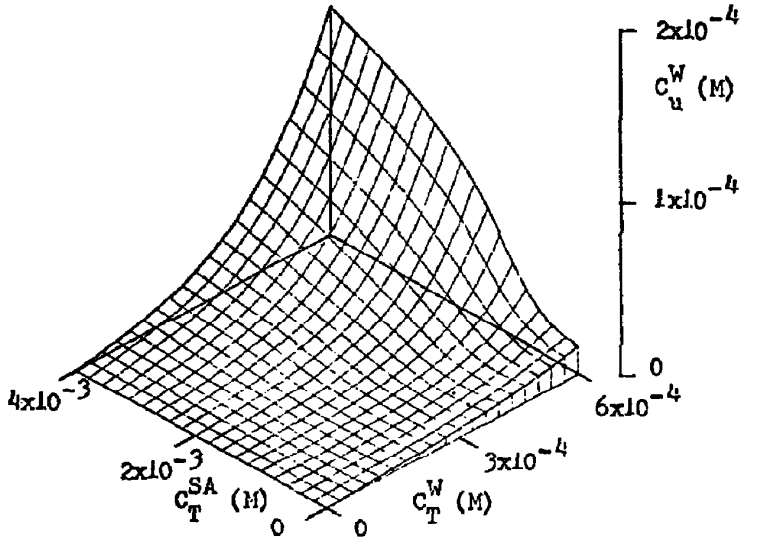

Figure 3.

Three-dimensional plot of unbound warfarin concentration versus total warfarin concentration and total salicylate concentration. Total protein concentration 5.4×10^{-4}M.

salicylate are shown in figure 2. There is considerable displacement of warfarin at the higher concentrations of added salicylate; displacement of salicylate by warfarin was also observed. The apparent intercept on the r-axis for warfarin in the presence of salicylate is less than that for warfarin by itself. One is therefore tempted to assign this change to a decrease in the number of binding sites on albumin. A similar, but erroneous conclusion would have been drawn from the simulations, shown in figure 1, if they had not been extended to sufficiently low values of r/c. Thus, without more extensive studies it cannot be said that salicylate decreases the number of binding sites for warfarin on albumin. The data can be more appropriately and conveniently represented by a three-dimensional plot of the unbound warfarin concentration versus the total concentration of warfarin and the total concentration of salicylate. The protein concentration would add a fourth dimension but in most situations remains relatively constant. The plot displayed in figure 3 was obtained after fitting equation (4) to the data, using a non-linear least-squares fitting routine; the number of terms chosen was the minimum that satisfactorily fitted the data. The displacing effect of one drug upon another is readily seen in this form of representation. We would stress that this approach is one simply of representation and not interpretation, in that no significance is placed on the model used or the associated parameters. However, the use of a binding isotherm such as equation (4) and its three-dimensional representation allows one to more readily visualize drug displacement interactions than the Scatchard plot and the like, and one, we believe, that is applicable in a variety of situations: particularly pharmacokinetic modelling.

We would like to thank Dr. W.G. Bardsley for some useful discussion concerning this topic.

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