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Phenylbutazone Plasma Binding: Effects of Salicylic Acid, Indomethacin, and Dicloxacillin (40700)

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Virtually all drugs and substances that are highly bound to human serum albumin are potentially capable of displacing each other from their binding sites in the protein molecule. This type of drug interaction can influence significantly the therapeutic and toxic effects of the competing drugs. Phenylbutazone, a nonsteroidal anti-inflammatory drug, has been shown to displace anticoagulants, antidiabetics, and antibiotics from their binding sites in human serum albumin resulting in enhanced therapeutic or toxic effects of these displaced drugs (1–4). Conversely, albumin-bound phenylbutazone can also be displaced by other drugs and substances such as anticoagulants, antidiabetics, and fatty acids (5, 6). Despite the enormity of possible drug interactions involving protein binding, however, relatively few studies have been done to investigate drug binding competition. In this report, we examine the effects of other highly bound drugs (salicylic acid, indomethacin, and dicloxacillin) on the binding of phenylbutazone to human plasma proteins and discuss the clinical significance of these drug binding interactions.

Materials and methods. Phenylbutazone, salicylic acid, and indomethacin were obtained from Sigma Chemical Company, St. Louis, Missouri, while sodium dicloxacillin was obtained from Bristol Myers Company, Syracuse, NY. Whole human plasma was provided by the blood bank of Ramathibodi Hospital, Bangkok, Thailand.

The binding of phenylbutazone to human plasma proteins was investigated using the equilibrium dialysis technique as described by McMenamy (7) with some modifications as follow: Cellulose dialysis tubing (Fischer Co., Pittsburg, Pa.) was soaked in distilled water for 3 days with water changes twice daily. After the washing, inflated dialysis bags with glass spacers inside were prepared and dried. Protein solution (human plasma) was pipetted into the dialysis bags and the bags were inserted into test tubes containing 0.1 *M* phosphate buffer, pH 7.4, with various concentrations of phenylbutazone. The test tubes were stoppered and attached to a multipurpose rotator (Model 15 V, Scientific Industries, Inc., Springfield, Mass.) with an inclination of about 30°. Dialysis was done at 4°C for 24 hr. Preliminary experiments showed that equilibration was complete within 24 hr and that there was no significant binding to the dialysis bag or to the glass. Phenylbutazone concentrations in the inside and outside solutions were determined using the spectrophotometric method described in detail by Burns *et al.* (8). The extent of binding was calculated from the following formula:

$$\% \text{ Binding} = \frac{C_t - C_f}{C_t} \times 100, \qquad [1]$$

where C_t = total phenylbutazone concentration (inside the bag), and C_f = concentration of free phenylbutazone (outside the bag).

The results obtained by the technique of putting the drug in the inside solution were essentially the same as that of putting the drug in the outside solution. The latter technique, however, was chosen in our experiments to avoid dilution of the protein solution and because it was easier to perform.

Competition with phenylbutazone plasma binding was studied by adding a constant concentration of the competitor drug to the outside solution of the dialysis tubes. Salicylic acid and indomethacin were dissolved in ethanol while sodium dicloxacillin was dissolved in distilled water. Preliminary experiments showed that ethanol, in the strengths used (3.5–7.0%), did not interfere with phenylbutazone plasma binding.

The effect of salicylic acid on the binding of phenylbutazone to 3.5% human serum albumin (Sigma Chemical Co.) was also deter-

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mined by equilibrium dialysis and the binding data were analyzed using the Scatchard plot (9).

Results. Displacement of phenylbutazone from its binding sites in human plasma was observed with salicylic acid and indomethacin while there was no apparent change with dicloxacillin (Fig. 1). In a wide range of total plasma phenylbutazone concentrations, the percentage binding of phenylbutazone was decreased when either salicylic acid or indomethacin was added. At a total salicylic acid concentration of about 200 μg/ml (which is within the range of therapeutic concentrations for anti-inflammatory effects), the percentage plasma binding of phenylbutazone was decreased by about 4–14% resulting in a two- to fourfold increase in the concentration of the free drug. With indomethacin concentration of about 200 μg/ml, which is extremely beyond the range of therapeutic concentrations obtained clinically (10), the decrease in phenylbutazone plasma binding was

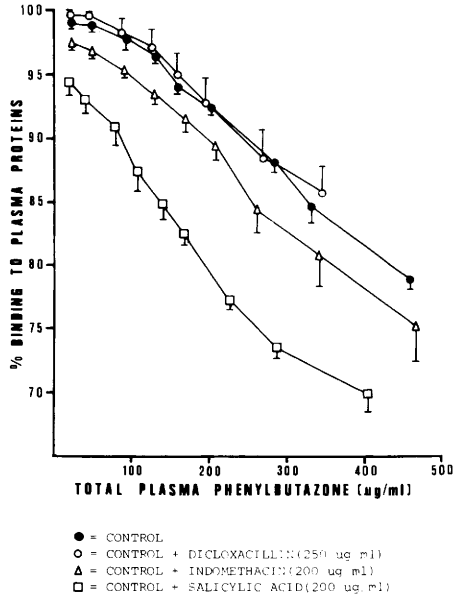

FIG. 1. Plasma protein binding of phenylbutazone in the presence of dicloxacillin, indomethacin, and salicylic acid. The binding of phenylbutazone to plasma proteins in the presence of salicylic acid (200 μg/ml), indomethacin (200 μg/ml), and dicloxacillin (250 μg/ml) was determined by equilibrium dialysis as described under Materials and Methods. The percentage of bound phenylbutazone was plotted against the total phenylbutazone concentration in the plasma. Each point represents the mean ± SE of three separate experiments.

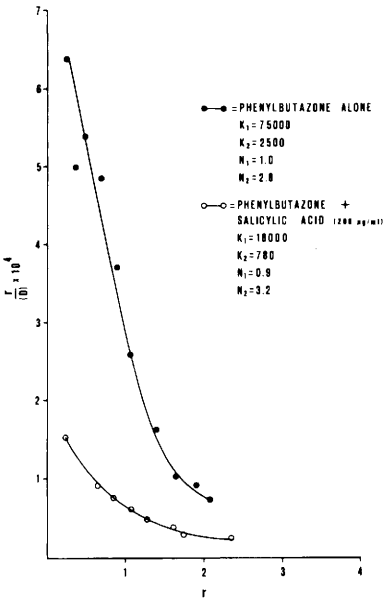

FIG. 2. Effect of salicylic acid on the binding of phenylbutazone to 3.5% human serum albumin. The binding data were obtained by equilibrium dialysis and analyzed according to the Scatchard plot: r = moles phenylbutazone per mole of albumin, (D) = concentration of free phenylbutazone, K = affinity constant, N = number of binding sites. Subscripts 1 and 2 refer to the high-affinity and low-affinity types of binding sites, respectively.

about 2–4%. Dicloxacillin, even at a very high concentration of about 250 μg/ml, did not significantly alter the extent of phenylbutazone plasma binding.

The effect of salicylic acid on the binding of phenylbutazone to 3.5% human serum albumin is shown in Fig. 2. The affinity constants (K_1 and K_2) of phenylbutazone as estimated by the Scatchard plot were dramatically reduced in the presence of salicylic acid (200 μg/ml) while there was no apparent change in the number of binding sites (n_1 and n_2).

Discussion. The displacement of protein-bound phenylbutazone caused by salicylic acid has important clinical implications because the concentration (200 μg/ml) used in this experiment could be easily reached with usual doses of salicylates in the treatment of rheumatoid arthritis and related diseases to achieve antiinflammatory effects. This interaction may enhance the toxic effects of phenylbutazone and salicylates when these drugs are combined in the therapy of arthritic dis-

eases. Although the decrease in the percentage binding is only 4–14%, the resulting increase in the concentration of the free drug is greatly magnified (two- to fourfold). Even if phenylbutazone is given at one-half the usual dose, when combined with salicylates, toxic levels of the free drug could still be reached while there may be actually a decrease in the total plasma concentration.

On the other hand, the slight displacement caused by indomethacin is not likely to be of clinical importance because this displacement was observed at an extremely high concentration of indomethacin not encountered clinically. Surprisingly, dicloxacillin did not appear to affect the plasma binding of phenylbutazone. This indicates that highly bound drugs do not necessarily displace each other from their binding sites in the protein molecule. A possible explanation is that they are bound at different mutually noninteracting sites in the albumin molecule.

The binding competition between salicylic acid and phenylbutazone is further elucidated by experiments using human albumin. Both the high affinity constant (K_1) and the low affinity constant (K_2) were decreased while the number of binding sites (n_1 and n_2) remained practically unchanged (Fig. 2). This indicates that the two drugs compete for the same binding sites in the albumin molecule.

Summary. The effects of three highly bound acids, viz salicylic acid, indomethacin, and dicloxacillin, on the binding of phenylbutazone to human plasma proteins were studied by equilibrium dialysis. Salicylic acid was found to displace phenylbutazone from its binding sites to a degree which might be clinically significant. Indomethacin caused a slight decrease in the percentage binding of phenylbutazone which is not likely to be of clinical importance. Dicloxacillin, however, did not appear to affect the plasma protein binding of phenylbutazone suggesting that the two drugs could be bound at mutually noninteracting sites in the protein molecule.

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