Rate of Binding of Antibiotics to Canine Serum Protein

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The time rates of binding of three antibiotics of similar chemical structure, each with differing degrees of protein binding, were determined. Cephaloridine, which is 10% bound by serum proteins, was bound at a more rapid rate than cephalothin, which is 40% bound by serum protein. Cefazolin, bound 80%, required for longest time period for maximum binding to occur. The rate of protein binding appears directly related to the total percentage bound. The data from this study indicate that prolonged rates of binding of highly protein-bound drugs may influence pharmacological studies.

Intravenous and intramuscular administration of cefazolin, a semisynthetic derivative of cephalosporin C, yields serum antibiotic levels that greatly exceed those attainable with equivalent doses of cephalothin or cephaloridine (5, 7, 9, 10). Cefazolin has been reported to be 70 to 90% bound by serum proteins (2, 4, 5). Cefazolin would therefore be bound to a greater extent than either cephaloridine, which is reported to be 5 to 30% protein bound, or cephalothin, which is reported to be 40 to 70% protein bound (1, 4, 6, 10, 11).

Recent studies in our laboratories have shown that between 70 to 80% of cefazolin is bound by serum protein in dogs. Cephaloridine was 10% protein bound and cephalothin was 40% protein bound in the same dogs. It was also found that, soon after administration of cefazolin, levels were extremely high when compared to equivalent doses of cephaloridine and cephalothin. It was felt that the discrepancy between these two findings might in part be explained by differences in the rates at which antibiotics were bound to canine serum proteins. The following studies were performed to explore that possibility.

MATERIALS AND METHODS

Pooled dog serum (1500 ml) was divided into three equal portions. The pooled dog serum had a total protein concentration of 6.8 g/100 ml (normal = 5.4 to 7.1 g/100 ml). The albumin was 3.4 g/100 ml (normal = 2.3 to 3.2 g/100 ml), and the globulin was 3.4 g/100 ml (normal = 2.7 to 4.4 g/100 ml). Cephaloridine, cephalothin, and cefazolin were added to each of three 500-ml samples of pooled serum in quantities sufficient to provide a final concentration of 20 ug/ml. These samples were incubated at 37 C. Samples (25 ml) of each of the three were removed at 10-min intervals during the 1st h of incubation, and a final specimen was obtained at 16 h. Each sample was immediately subjected to ultrafiltration. Ultrafiltration was performed by using a Visking membrane in a vacuum of - 40 cm of Hg for 2 min to obtain a volume of protein-free fluid of 0.5 ml. The very low pressure and brief time of filtration were employed to reduce the possibility that a significant degree of dissociation of the protein-bound antibiotics would occur, thereby yielding spurious results.

A cylinder-plate bioassay employing Sarcina lutea as the test organism was employed for the determination of antibiotic levels. Known quantities of cephaloridine, cephalothin, and cefazolin were prepared in phosphate-buffered 0.15 M saline at pH 6.0 and employed as controls. The concentration of free antibiotic in each sample was determined after ultrafiltration of the serum and control solutions.

The experiment was repeated five times, and changes in concentration of free antibiotic during the time periods described above were determined for cephaloridine, cephalothin, and cefazolin by an analysis of variance.

RESULTS

Cephaloridine was 5% protein bound within 10 min and reached a maximum of 10% protein binding in 20 min. (Fig. 1). Cephalothin was 12% bound in 10 min, 34% bound in 20 min, and reached a maximum of 40% protein binding by 30 min. Cefazolin was 6% bound in 10 min, 24% bound in 20 min, and was bound to a maximum of 80% in 50 min.

The rate of binding of all three antibiotics appears to be linear in relation to the duration of exposure to protein from the time of mixing and incubation until the maximum percent of binding for each antibiotic occurs. It also appears that the rate of binding of the three antibiotics studied is directly related to the total percentage bound. The greater the per-
per centage of total antibiotic bound, the longer the time period required for this to occur. Analysis of variance showed the differences in each antibiotic's rate of binding to be highly significant ($P > 0.01$).

**DISCUSSION**

Previous studies have shown that different antibiotics exhibit different affinities for serum proteins and combine to varying degrees, and an equilibrium between the protein-bound and -free antibiotic occurs (3, 8). The quantity of free antibiotic present in the serum is dependent upon a number of factors. The degree of binding is dependent upon the animal species, and within each species the serum concentration of an antibiotic is dependent upon the quantity of protein and antibiotic present, the affinity of antibiotic and protein for each other, the type of protein and its binding sites, the rate and degree of binding and dissociation, and the techniques employed in measuring binding.

This study shows that cefazolin is bound to a maximum of 80% by dog serum proteins, was only bound 7% in 10 min, and required 50 min before maximum binding occurred. Cephalothin and cephaloridine, antibiotics of similar chemical structure, were maximally bound within 20 min. The percent binding of all of the cephalosporins tested did not increase or decrease after 1 h.

In vitro and in vivo protein binding of an antibiotic may differ, and the effect of protein binding on therapeutic effectiveness of an antibiotic is not known; consequently, no profound conclusions can be drawn from these factors. However, it would appear that the rate of binding and dissociation of an antibiotic will have an effect on concentrations of free antibiotic available in serum and in body fluids containing varying quantities of protein.

A slow rate of protein binding for an antibiotic may result in "falsely" elevated serum levels, if specimens are run before the time necessary for maximum binding has elapsed. This might influence values used in determining half-life, distribution, excretion, and metabolism of antibiotics whose concentrations are measured by bioassay techniques. These factors should be considered in future biopharmakinetetic studies of compounds that are highly protein bound.

**LITERATURE CITED**


