Serum Protein Binding of the Aminoglycoside Antibiotics

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The binding of four aminoglycoside antibiotics by human serum was investigated under controlled conditions of physiological pH and temperature, by means of an ultrafiltration technique. No serum binding was demonstrable for gentamicin, tobramycin, or kanamycin, whereas streptomycin was 35% bound. Previous conflicting studies are discussed, and some of the pharmacological implications are considered.

Serum protein binding is a subject of pharmacological and clinical interest because it affects the distribution, therapeutic activity, toxicity, and excretion of drugs (12). The primary mechanism for excretion of the aminoglycoside antibiotics is glomerular filtration (14), and the amount filtered is proportional to the concentration of unbound drug (16). Previous workers have reported values ranging from 0 to 30% for the binding of gentamicin, but no information is available for tobramycin, the newest member of the aminoglycoside group of antibiotics. The present study was carried out to gain further information regarding the protein binding characteristics of these important antibiotics and to compare the results with those for kanamycin and streptomycin.

MATERIALS AND METHODS

The binding of gentamicin, tobramycin, kanamycin, and streptomycin by undiluted pooled human serum obtained from healthy volunteers was investigated under controlled conditions of physiological pH and temperature by use of an ultrafiltration method previously described (3). The concentrations of antibiotics used were those commonly found in the blood during therapy: 5 μg/ml for gentamicin and tobramycin, 5 and 15 μg/ml for kanamycin, and 15 μg/ml for streptomycin. Pooled serum from healthy donors containing the antibiotic under study was adjusted to a pH of 7.4 to 7.5 by bubbling 5.0% CO₂ through it. Then 15.4 ml of this 97% serum solution was put in the upper of two glass chambers, which were clamped tightly with a single layer of cellophane membrane (Union Carbide Corp., Dialysis Membrane 1-36) between them. Ultrafiltration was carried out in an incubator at 37 C by the application of a vacuum to the lower chamber, and yielded 0.8 ml of filtrate within 45 min. By removing 1.0 ml from the upper chamber when 0.4 ml of filtrate had been collected, a midpoint specimen was available for antibiotic assay which corresponded to 100% serum because of the loss of water during filtration. The concentrations of antibiotic in this midpoint serum specimen, and in the protein-free ultrafiltrate, were determined by use of an agar well assay method with Bacillus subtilis ATCC 6633 as the test organism (2). Appropriate standard curves for the microbiological assay were prepared by dissolving known amounts of the antibiotic standards in fresh, pooled human serum and in antibiotic-free filtrate obtained by filtration of pooled serum. Impermeability of the cellophane membrane as a cause of apparent protein binding was investigated by ultrafiltration of buffered saline solutions of the antibiotics (6). The antibiotic standards used in these studies and their suppliers were gentamicin (Schering Corp.), tobramycin (Eli Lilly & Co.), kanamycin (Bristol Laboratories), and streptomycin (Canalco).

RESULTS

Percent binding was calculated by dividing the difference between the antibiotic concentrations in the serum and filtrate specimens by the serum concentration and multiplying by 100 (3). The numbers of separate ultrafiltrations performed and averaged were 9 for gentamicin, 8 for tobramycin, 12 for kanamycin at 5 μg/ml, 6 for kanamycin at 15 μg/ml, and 6 for streptomycin. These ultrafiltration experiments with gentamicin and tobramycin in 100% human serum demonstrated no evidence of protein binding, with mean values of -2.0 and -2.1%, respectively (Table 1). Kanamycin experiments yielded values of -2.8% at 5 μg/ml and -0.7% at 15 μg/ml, whereas streptomycin was 35.4% bound at a serum concentration of 15 μg/ml. Membrane trapping as a cause of apparent protein binding was found to be negligible (retention <3.0%) as determined by ultrafiltration of buffered saline solutions of the antibiotics. The slight fluctuations
around zero of the protein binding figures for gentamicin, tobramycin, and kanamycin appeared to be attributable to variations inherent in the biological assay system, because the mean binding percentages did not differ significantly from zero as determined by the t test \((P > 0.3)\).

**DISCUSSION**

Under conditions simulating those that occur in the human body, these ultrafiltration studies show that the serum protein binding of gentamicin and tobramycin is essentially zero. Previously reported figures for serum binding of gentamicin, obtained by a variety of methods, have ranged from 0 to 30\% (5, 13, 17, 21). In studies in which cellophane membrane techniques were used, the antibiotic concentrations used were very large (21), or the methods employed were not described in sufficient detail to compare them with ours. Naumann and Auwärter, using an agar diffusion method with clinically attainable serum concentrations of 2.5 and 5.0 \(\mu\)g/ml, found, as we have, no evidence of serum protein binding of gentamicin (13).

A good correlation has been observed by some investigators between protein binding and a decrease in antibacterial activity in the presence of serum with a number of antibiotics (11, 18). The influence of serum on the antimicrobial activity of gentamicin has been studied by several workers, but the results are difficult to evaluate because of the techniques employed. Rubenis and associates (19) concluded that serum “decreased the bactericidal rate and effect of gentamicin” against *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* on the basis of tests demonstrating better early killing in 10\% as compared to 80\% serum; results in broth were not presented. A three- to fourfold increase in the minimal inhibitory concentration in 50\% horse serum was reported for *S. aureus* and *P. aeruginosa* by Weinstein (21), and an eightfold increase against a single strain of *S. aureus* was found by Barber and Waterworth (1) in bacteriostatic tests in 90\% pooled human serum; however, difficulties in reading end points in cloudy solutions may have influenced both of these studies. Klein and associates (10), using bactericidal end points, found essentially no effect with 50\% human serum versus broth for *S. aureus*, *B. cereus*, and *Sarcina lutea*. More recently, Davis and Ianetta (8) observed a marked decrease in activity of gentamicin against most strains of *P. aeruginosa* in the presence of 20\% serum, which was attributable to the calcium present rather than to protein binding. With other gram-negative bacilli, serum did not inhibit the activity of gentamicin. Thus, the evidence concerning the inhibition of gentamicin activity by serum in broth dilution studies is conflicting, and does not permit definite conclusions in regard to protein binding.

Utilizing a different approach, Riff and Jackson (17) studied the distribution of radioactive gentamicin (100 \(\mu\)g/ml) in whole blood and plasma to which autologous erythrocytes were added. After centrifugation, which removed the erythrocytes and 10\% of the radioactivity, plasma proteins were precipitated by addition of trichloroacetic acid, which removed another 30\% of the radioactivity. These results were interpreted as demonstrating 30\% serum protein binding of gentamicin; however, no details were given about the effect of trichloroacetic acid on this basic antibiotic itself. Other factors to be considered are the possible effects of the trichloroacetic acid on the protein binding sites and the high concentration of gentamicin used, which is not in the usual clinical range.

No evidence of protein binding of kanamycin by human serum was demonstrable in the present study which confirms reports of other workers who used a variety of techniques (15, 20, 22). Our finding of 35.4\% binding of streptomycin agrees closely with the previously published range of 30 to 35\% noted by other investigators (4, 20).

The presently reported finding of essentially no
protein binding of gentamicin is compatible with the pharmacological studies of Cutler and co-workers (7, 9), who found no difference in the renal clearance of gentamicin and inulin, a substance excreted exclusively by glomerular filtration. To account for the widely accepted figure of 25% protein binding for gentamicin, these authors postulated that "net tubular secretion'' could explain the recovery of the aminoglycoside as if it were excreted entirely by glomerular filtration. Our finding of no protein binding of gentamicin would indicate that it is not necessary to invoke tubular secretion to explain the excretion of the drug.

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LITERATURE CITED