ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1991, p. 2232–2237
0066-4804/91/112232-06$02.00/0

Vol. 35, No. 11

Characteristics of Ceftriaxone Binding to Immunoglobulin G and Potential Clinical Significance

HE SUN,[1,2] MOSES S. S. CHOW,[2,3] AND EUFRONIO G. MADERAZO[1,4]*

Medical Research Laboratory and Infectious Diseases Division, Department of Medicine,[1] and Drug Information Center,[3] Hartford Hospital, Hartford, Connecticut 06115[3]; Departments of Medicine and Pathology, University of Connecticut School of Medicine, Farmington, Connecticut 06032[4]; and the University of Connecticut School of Pharmacy, Storrs, Connecticut 06268[2]

Received 1 April 1991/Accepted 7 August 1991

The interaction between immunoglobulin G (IgG) and ceftriaxone was studied. Using an ultrafiltration method, we performed dose ranging studies at a ceftriaxone concentration range of 1 to 720 μg/ml in the presence of various concentrations of human IgG, human serum albumin (HSA), and combinations of IgG and HSA at pH 7.4 and 37°C. The results showed that ceftriaxone binding to IgG was nonlinear and was consistent with the presence of two binding sites that possess different binding capacities and affinities. Except for increased peak percent binding as the IgG concentration increased, the binding characteristics did not change with IgG concentration. Binding to HSA was consistent, with the presence of only one high-affinity binding site. A mathematical model based on the observed data was constructed; this model was used to predict protein binding at various concentrations of drug, IgG, HSA, or combinations of IgG and HSA in buffer and in plasma medium. Correlations between the observed versus the predicted values were excellent in both media. Simulations with the model indicated that patients with hypergammaglobulinemia have an increased potential of being exposed to prolonged subinhibitory concentrations of ceftriaxone if the drug is given once every 24 h.

Increasing use of large doses of intravenous (i.v.) immunoglobulin G (IgG) necessitates a closer examination of drug binding to immunoglobulins to ascertain whether drug dosing regimens need to be altered to maintain drug effectiveness. Suspicion that certain drugs may bind to immunoglobulins has been heightened by a recent report showing that large doses of human IgG given in conjunction with ceftriaxone to neonatal rats infected with a group B streptococcus raised the mortality rate from 56.5% when ceftriaxone was used alone to 95.7% when they were used together (10). One potential explanation for this phenomenon is that IgG binds ceftriaxone. More recently, Cantu et al. (7) reported unusual vancomycin pharmacokinetics in a patient with IgA myeloma. Total concentrations of vancomycin in serum were extremely elevated, but the free fraction in serum was only 3%, while control samples from other patients receiving vancomycin were 62 to 90%. The elimination half-life was prolonged despite normal renal function, and vancomycin therapy was clinically ineffective. Extensive binding of vancomycin, presumably to high concentrations of the IgA protein, was suspected to account for these observations. The IgA subtype involved was not characterized.

To prove the hypothesis that some drugs may bind to immunoglobulins, a detailed in vitro kinetic evaluation was undertaken with IgG. Since ceftriaxone was the antibiotic used in the rat study noted above (10), that drug was chosen for this study. Moreover, cephalosporins are the most commonly used antibiotic drugs in hospitalized, infected patients, and therefore, they are the most likely antibiotic group to be used concomitantly with large i.v. doses of IgG.

The essential kinetic features of ceftriaxone are its extensive and saturable protein binding in the therapeutic range and elimination about equally by biliary secretion and by the kidney, the latter almost exclusively by glomerular filtration (2, 14, 24). Its elimination half-life is about 8 h (2, 25, 26). The relatively long half-life is at least partly a direct consequence of its substantial protein binding and the absence of renal tubular secretion (2, 25).

MATERIALS AND METHODS

Free concentrations of ceftriaxone (C_f) were determined by ultrafiltration (23, 30) by using the CETRIFREE micropartition system (Amicon Division, W. R. Grace & Co., Beverly, Mass.). Samples were equilibrated for 30 min at 37°C and were then centrifuged at 1,500 × *g* for 20 min. The free concentration was determined from the ultrafiltrate.

The ceftriaxone concentrations in the ultrafiltrate and in phosphate-buffered saline (PBS) were determined by the spectrophotometric method (model 250; Gilford Instrument Laboratories Inc., Oberlin, Ohio). The maximum absorption wavelength of ceftriaxone in PBS, ultrafiltered PBS, ultrafiltered IgG in PBS solution, and ultrafiltered albumin in PBS solution was 240 nm. At a concentration of 1 μg/ml, the within-day coefficients of variation were 0.36% in PBS buffer solution and 2.91% in ultrafiltered IgG or ultrafiltered albumin solutions. The overall day-to-day coefficient of variation was 4.5%, which was obtained for slopes of standard curves over a 4-month period. The ceftriaxone concentration in plasma ultrafiltrate was determined at a wavelength of 275 nm. At this wavelength, a plasma ultrafiltrate blank has a very low absorption. Drug-free samples were used as blank controls for all drug concentration determinations.

Preliminary experiments indicated that ceftriaxone does not bind to the filter membrane, regardless of whether PBS or plasma ultrafiltrate is used as the solvent medium. Consequently, PBS buffer was used as the solvent medium in subsequent experiments.

Binding of ceftriaxone to IgG and/or albumin. The binding

* Corresponding author.

kinetics of ceftriaxone to IgG were determined by adding the drug into the IgG solution which was prepared from i.v. IgG (Cutter Biological, Elkhart, Ind.) containing 50 g of protein per liter, of which no less than 98% of the protein was IgG. The solution was diluted with PBS, and the pH was adjusted to 7.4 just before the drug was added. Binding was studied at IgG concentrations of 12, 25, and 50 g/liter, using 7 to 11 different concentrations of ceftriaxone between 2 and 720 μg/ml for each fixed IgG concentration.

In addition, we studied the binding of ceftriaxone at a dosage range of 12.7 to 932 μg/ml to fatty acid- and globulin-free human serum albumin (HSA; Sigma Chemical Company, St. Louis, Mo.) at concentrations of 20 and 40 g/liter.

Ceftriaxone concentrations that varied from 12.7 to 932 μg/ml were used to study the binding of ceftriaxone to a mixture of 23 g of IgG per liter and 20 g of HSA per liter and were compared with binding in 20 g of HSA per liter alone, without IgG.

Binding of ceftriaxone to plasma with high levels of IgG. Human plasma samples from a healthy volunteer with known concentrations of HSA and total immunoglobulins were used. IgG and ceftriaxone were added to the plasma to obtain the desired concentrations. The correlation between the observed versus predicted free ceftriaxone concentrations was calculated.

Effects of pH and temperature on ceftriaxone binding to IgG. The effects of pH and temperature were tested with a fixed drug concentration of 10 μg/ml. We used a lower IgG concentration of 12.5 g/liter to study pH effects and a higher IgG concentration of 24.5 g/liter to study temperature effects, because pH usually has a greater effect on drug binding than temperature does. pHs were adjusted to 3.5, 4.0, 6.7, 9.6, and 10.9 by using 0.05 N HCl or 0.05 N NaOH. The effect of temperature was studied by incubating the samples at 6, 23, 37, and 40°C. Two controls were tested simultaneously; one contained drug in PBS to determine filter membrane binding, and drug-free IgG in PBS was used as the blank.

Binding kinetic parameters. Ceftriaxone binding parameters were estimated by nonlinear least-squares regression with SAS PROC NLIN (9). All the initial estimates were obtained from the Scatchard plot (6, 8). Parameters were obtained by the following equation (11, 20, 28):

$$\%C_b = \left[\frac{N_1 \cdot K_1 \cdot C_f}{(1 + K_1 \cdot C_f)(C_b + C_f)} + \frac{N_2 \cdot K_2 \cdot C_f}{C_b + C_f}\right] \cdot 100 \quad (1)$$

where $\%C_b$ is the percentage of bound drug; N_1 and N_2 are the maximum binding capacities of the two binding sites; K_1 and K_2 are the association constants of the binding sites; and C_f and C_b are free and bound drug in molar concentrations, respectively, in which C_b is calculated by subtracting C_f from the total concentration. For the low-affinity binding site, $N_2 \cdot K_2$ was determined because N_2 and K_2 cannot be determined separately from the experimental drug concentration range used (20, 28).

For ceftriaxone-HSA binding, a saturable single-binding-site model was used, according to equation 2 (11, 20, 28):

$$\%C_b = \frac{N_3 \cdot K_3 \cdot C_f}{(1 + K_3 \cdot C_f)(C_b + C_f)} \cdot 100 \quad (2)$$

where N_3 is the maximum binding capacity of HSA, K_3 is the association constant, and C_b and C_f are as defined above.

Prediction and simulation of free ceftriaxone concentrations in the presence of HSA and IgG. When both HSA and IgG are present, the system is complex, with free drug in equilibrium with bound drug on the three binding sites (two on IgG and one on HSA). The relationship between total drug concentration and free drug concentration can be described in equation 3 (11, 20, 28):

$$C_{total} = C_b + C_f = \frac{n_1 \cdot K_1 \cdot C_f \cdot P}{1 + K_1 \cdot C_f} + n_2 \cdot K_2 \cdot C_f \cdot P + \frac{n_3 \cdot K_3 \cdot C_f \cdot A}{1 + K_3 \cdot C_f} + C_f \quad (3)$$

where C_{total} is the total ceftriaxone concentration; n_1, n_2, and n_3 are the calculated binding capacities of the binding sites on the protein molecule (calculated by the maximum binding capacity [N_1, N_2, N_3] divided by protein molar concentration); P is the IgG molar concentration; and A is the HSA molar concentration. All other parameters are the same as defined above. Since equation 3 is a complex equation, it was difficult to calculate C_f directly from a known C_{total}. Consequently, the C_f for a specific C_{total} was calculated in a two-step manner by using a graphic method by first calculating the C_{total} values for a series of C_f values by using equation 3 and then plotting the C_f values versus the calculated C_{total} values. The C_f value for a particular C_{total} value could then be derived from the plot.

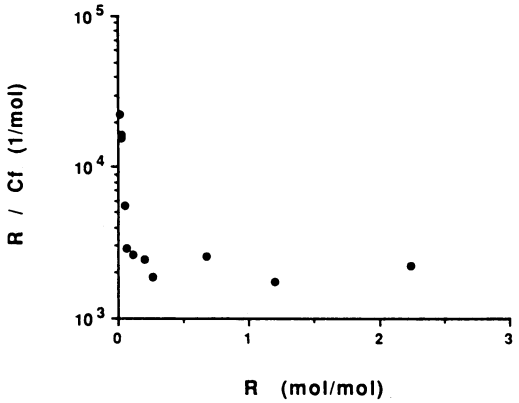

FIG. 1. Scatchard plot for ceftriaxone binding to IgG (12 g/liter). The datum points are the mean values of experiments performed in triplicate. R is the ceftriaxone concentration bound (in moles per liter)/IgG concentration (in moles per liter). The findings are consistent with the presence of at least two ceftriaxone-binding sites on the IgG molecule.

RESULTS

Binding to IgG in PBS. Unlike studies of ceftriaxone binding to plasma proteins or albumin in which only a single binding site was observed, the Scatchard plot (28, 29) of our studies with IgG indicated that there are two binding sites on the IgG molecule for ceftriaxone (Fig. 1). The two-binding-site model was the best model since additional parameters were not significantly different from zero with a three-binding-site model. The two-binding-site model was further confirmed with the F-ratio statistics and examination of residuals.

When the percent bound versus free drug concentration was plotted, the shape of the curve remained unchanged as the concentration of IgG was increased from 12 to 50 g/liter (Fig. 2). One of the two binding sites had a higher affinity but a very low binding capacity, whereas the other binding site had a very low affinity and a very high binding capacity.

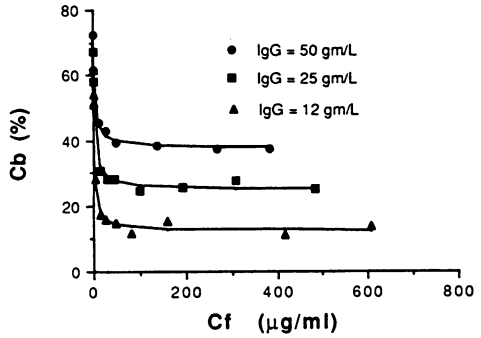

FIG. 2. Relationship of percent bound fraction (C_b) to free concentration (C_f) at various IgG concentrations in the medium. Each datum point is the average of triplicate experiments. Hypothetical curve-fitting lines were drawn by using equation 1 (see text).

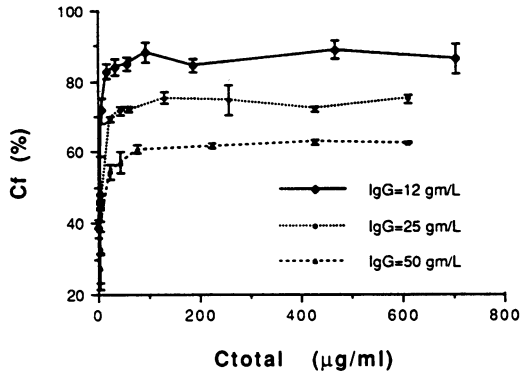

FIG. 3. Percent free drug as a function of the total drug concentration. The datum points are means ± standard deviations of three experiments performed in triplicate. The percent free fraction (C_f) rose as the total drug concentration (C_{total}) increased and reached a plateau above C_{total} of 100 μg/ml. The peak percent C_f decreased as the IgG concentration increased.

Table 1 summarizes the binding kinetic parameters. A plot of percent C_f as a function of C_{total} indicated that the C_f fraction is concentration dependent up to a C_{total} of approximately 100 μg/ml; above that, the C_f fraction reached a plateau and was concentration independent (Fig. 3). Figure 3 also shows that peak C_f fraction decreased as the IgG concentration increased.

Binding to albumin in PBS. A Scatchard plot of ceftriaxone binding to albumin by using 20 and 40 g of HSA per liter showed a linear relationship, suggesting the presence of a single binding site on the albumin molecule (data not shown). Similar to that which was observed with IgG in Fig. 3, the percent C_f increased directly with the C_{total} (Fig. 4), but a plateau was not reached within the experimental ceftriaxone concentration range used. The maximum binding capacity (N_3) was found to be 1 mol of drug per mol of albumin, and the association constant (K_3) was 2.5×10^4 M^{-1}. These values are in close agreement with those reported previously (3, 4, 17, 18, 22, 27).

Binding in the presence of IgG and albumin in PBS. In the presence of both HSA and IgG in PBS buffer, the observed C_f was similar to that predicted by equation 3. The correlation coefficient (r) of the predicted versus the observed values was 0.99, with a slope of 0.97 (versus a slope of 1, $P > 0.7$) (Fig. 5).

C_f was significantly decreased in the presence of both HSA and IgG (20 and 23 g/liter, respectively) when it was compared with the C_f in HSA (20 g/liter) alone ($P < 0.01$; paired t test). This effect was concentration dependent, and the mean decrease in C_f was 10.2 ± 6.5% over the experimental concentration range used.

By using equation 3 simulation, the effect of hyperimmunoglobulinemia G can be studied. Under this condition, the change in percent C_f as a function of C_{total} is biphasic. Thus, comparing C_f in 10 g of IgG per liter (normal) with C_f in 50 g of IgG per liter, with the albumin concentration fixed at 20 g/liter, a plot of the difference in percent C_f [$(C_{f\text{-normal IgG}} - C_{f\text{-high IgG}}) \times 100/C_{f\text{-normal IgG}}$], shows that the effect is least when C_{total} is about 100 μg/ml and higher at the lower and higher values (Fig. 6).

C_f increased directly with the pH up to pH 6.8. Beyond pH 6.8, the binding remained constant, as shown in Fig. 7. Similarly, the C_f fraction also increased directly with the temperature. The curve was also nonlinear, and the change from 35 to 40°C was minimal (Fig. 8). Thus, these effects are not expected to be clinically significant within the narrow pH and temperature ranges in humans.

Binding in plasma with high concentrations of IgG. In studies in which PBS is used as the medium, the effects of other plasma or serum components, particularly other nor-

TABLE 1. Ceftriaxone-IgG binding kinetic parameters[a]

IgG concn (g/liter)	N_1 (10^{-6} M)	K_1 (10^5 M^{-1})	$N_2 \cdot K_2$ (M/M)	n_1 (M/M)	$n_2 \cdot K_2$ (M^{-1})
12	2.23	12.7	0.140	0.031	1,943
25	4.26	7.23	0.326	0.029	2,216
50	4.40	7.90	0.598	0.015	2,036
Mean ± SD		9.27 ± 2.9		0.025 ± 0.009	2,065 ± 139

[a] N_1 and N_2, maximum binding capacities of the two binding sites of IgG; M, molar concentration, or moles per liter; K_1 and K_2, association constants of the two binding sites; n_1 and n_2, binding capacities for drug (moles per liter) of the two binding sites per IgG (moles per liter).

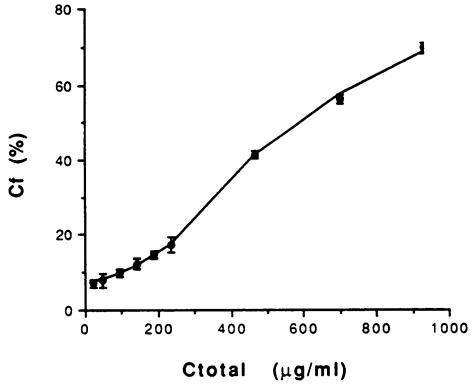

FIG. 4. Ceftriaxone binding to human serum albumin (40 g/liter) in PBS buffer solution. The datum points are means ± standard deviations (n = 3). The slightly sigmoidal curve differs from that observed with binding to IgG shown in Fig. 3. C_f, free concentration of ceftriaxone.

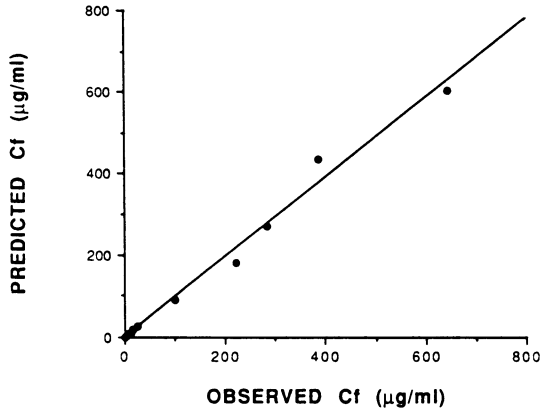

FIG. 5. Predicted versus observed free drug concentration by using PBS as the medium. Serum albumin and IgG concentrations were 20 and 23 g/liter, respectively. The datum points are means of triplicate experiments (slope = 0.97; r = 0.99).

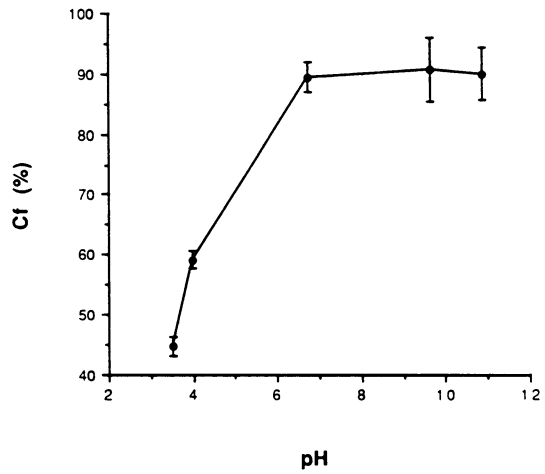

FIG. 7. Effect of pH on free drug concentration. The total ceftriaxone concentration used was 10 μg/ml, and IgG was used at 12.5 g/liter. Datum points are means ± standard deviation (n = 3).

mal proteins, are not taken into account. To study their potential influence on the binding of ceftriaxone to IgG and albumin and to simulate the in vivo situation, the study was repeated in normal human plasma. The levels of albumin and IgG were predetermined in the plasma samples, and exogenous human IgG was added to the desired concentrations.

The results showed that the model for the combined presence of IgG and albumin (equation 3) also fits the observed data. Linear regression obtained an excellent correlation between the predicted versus the observed C_f values (r = 0.998), as shown in Fig. 9. The slope of 0.81, however, was significantly different from 1 (p = 0.002), probably indicating the influence of additional, unknown factors in plasma.

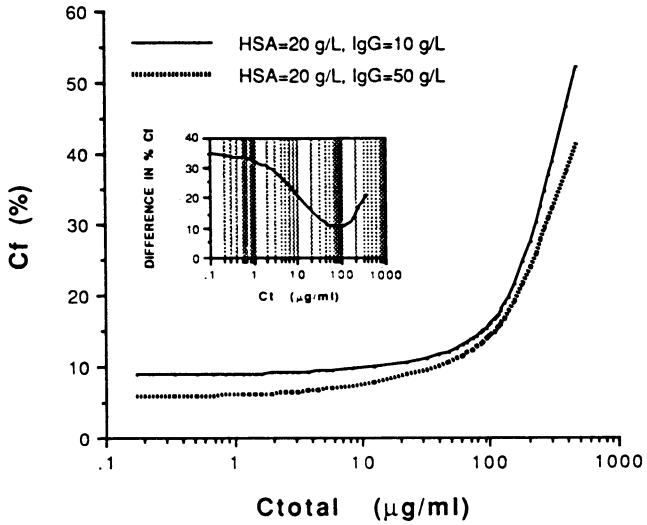

FIG. 6. Simulation of changes in percent free drug concentration (C_f) with total drug concentration (C_{total} and C_t [inset]) in the presence of 20 g of albumin per liter or 20 g of albumin per liter plus 50 g of IgG per liter. The following values for the parameters were used: n_1 = 0.025 M/M, $K_1 = 9.27 \times 10^5\ M^{-1}$, $n_2 \times K_2 = 2065.5\ M^{-1}$, n_3 = 1 M/M, and $K_3 = 2.5 \times 10^4\ M^{-1}$.

DISCUSSION

Although the binding of ceftriaxone to each specific plasma protein is not well characterized, its binding behavior to plasma (or serum) proteins, in general, is well studied (3, 4, 17, 18, 22, 27). When determined by equilibrium dialysis, the free fraction in plasma or serum increased from 4 to 44% over the drug concentration range of 0.5 to 600 μg/ml. A single binding site with a binding association constant of about $3 \times 10^4\ M^{-1}$ was described for plasma proteins or albumin (3, 4, 17, 18, 22, 27). In our study, similar results were obtained by using an ultrafiltration method. There was close agreement in the binding parameters in pooled human plasma protein and serum albumin, indicating, in fact, that albumin is the major binding protein in normal human plasma.

A previous study has shown that ceftriaxone does not bind avidly to α_1-acid glycoprotein at a glycoprotein concentration of 200 mg/dl (17). The binding characteristics, however, were not studied in detail.

Results of our present study show that ceftriaxone does, in fact, bind to IgG and that it has two binding sites on the IgG molecule. One site had a higher binding affinity than that for

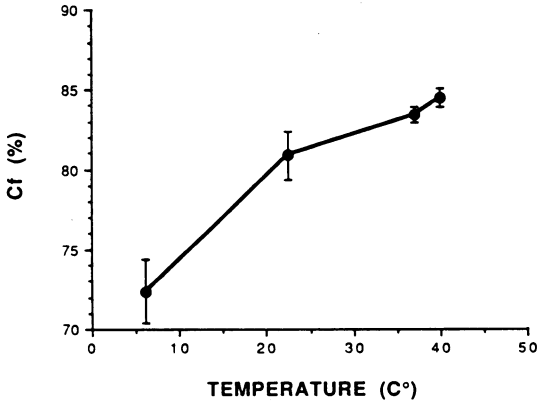

FIG. 8. Effect of temperature on free drug concentration (C_f). The total ceftriaxone concentration used was 10 μg/ml, and the IgG concentration was 24.5 g/liter.

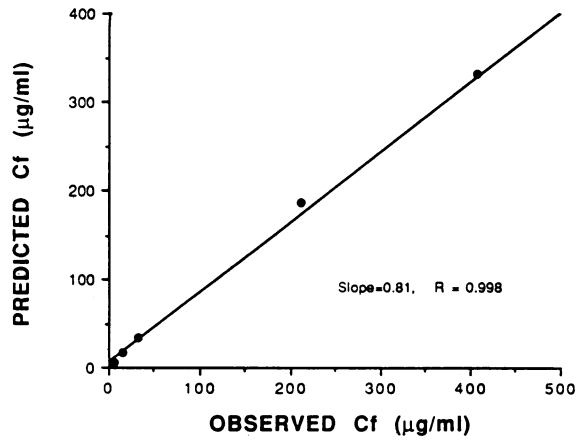

FIG. 9. Predicted versus observed free drug concentration (C_f) in human plasma medium. The albumin concentration was 20 g/liter, and the total IgG (endogenous and exogenous IgG) concentration was 34 g/liter. Datum points are means of two experiments.

albumin (9.3×10^5 M^{-1} versus 2.5×10^4 M^{-1} for albumin) but a much lower binding capacity than that for albumin (0.03 mol of drug per mol of IgG versus 1 mol of drug per mol of albumin). The other binding site had a low affinity but a high capacity. From these findings, it can be expected that at very low drug concentrations, ceftriaxone preferentially binds to the higher-affinity IgG binding site; once this higher-affinity IgG binding site is saturated, drug is then mostly bound to albumin and the higher-capacity IgG binding site. Thus, the effect of IgG on the free concentration of ceftriaxone is greater as the total concentration of the drug declines and as it increases to very high levels.

Binding is also IgG dose dependent, so that at concentrations of 10 g/liter (high limit of the normal IgG concentration in serum), the maximum proportion of bound drug is less than 15%, whereas at the 50 g/liter maximum, binding approaches 40%. In the presence of normal levels of serum albumin and IgG, binding to IgG is of minimal significance. When the IgG concentration is increased, however, as would be the case following massive i.v. dosing for certain indications (5, 15, 16, 19, 31), binding to IgG may assume potential clinical importance. This effect will be enhanced further in the presence of hypoalbuminemia.

The mechanism of drug binding to gamma globulins is not well studied (1). Previous studies have shown that isolated L chains have a low affinity for binding chemical compounds, whereas H chains have higher affinities (1, 21). It is possible, then, that this finding may explain the characteristics of the two apparent binding sites in IgG for ceftriaxone observed in this study. These sites should be nonspecific and, therefore, may be common to immunoglobulins belonging to the same type.

The very essence of immunoglobulin function is the ability to specifically bind foreign molecules. Thus, it is reasonable to anticipate that another mechanism of drug binding to immunoglobulin is through its antigen-binding site in the Fab fragment. This should be a relatively rare occurrence, however, because drugs are small molecules and molecules with molecular weights of <10,000 are generally not effective immunogens, although there are exceptions. Certain peptides with molecular weights of about 4,500 to 5,000, such as glucagon and insulin, may induce antibody formation. When combined with a protein carrier (hapten), even very low molecular weight drugs may become potent immunogens. The case mentioned above that showed the unusually high protein binding of vancomycin in a patient with immunoglobulin A myeloma (7) could be an example of this possibility, but this was not specifically studied.

TABLE 2. Time after dose of ceftriaxone[a] at which free ceftriaxone concentration in serum was maintained above 0.5 or 1.0 μg/ml at different IgG doses and IgG concentrations in serum[b]

Single dose of IgG given i.v. (mg/kg)	Peak IgG concn (g/liter)[c]	Estimated mean time (h) at which the free ceftriaxone was greater than:	
		0.5 μg/ml	1.0 μg/ml
0	10	15.3	9.0
100	12	14.7	8.8
500	20	14.0	8.2
1,000	30	13.0	7.5
400 (daily for 5 days)	35[d]	12.5	7.0
1,000 (weekly)	50[d]	10.8	6.0

[a] The ceftriaxone dose was 1 g given i.v.

[b] Simulation of free ceftriaxone concentrations in serum after various i.v. IgG dosages by using a two-compartment model for ceftriaxone, with a distribution half-life of 1.7 h and an elimination half-life of 8 h, were used. Binding parameters were as follows: $n_1 = 0.025$ M/M, $K_1 = 9.27 \times 10^5$ M^{-1}, $n_2 \cdot K_2 = 2065.5$ M^{-1}, $n_3 = 1$ M/M, and $K_3 = 2.5 \times 10^4$ M^{-1}, where n_1 and n_2 are the binding capacities of the two binding sites on IgG, n_3 is the binding capacity of the single binding site on HSA, K_1 and K_2 are the association constants of each IgG binding site, and K_3 is the association constant of the HSA binding site.

[c] Values were obtained from references 15, 16, 19, and 31.

[d] Peak concentration after the fifth dose.

From a clinical perspective, assuming a dose of 1 g of ceftriaxone given i.v., a two-compartment model for ceftriaxone with a distribution half-life of 1.7 h, an elimination half-life of 8 h, and a volume of distribution-protein binding relationship described by McNamara et al. (12, 13), the effective free drug concentration would be below 1 μg/ml approximately 3 h earlier or below 0.5 μg/ml 4.5 h earlier in a patient with high levels of IgG (50 g/liter) in serum than it would be in the same patient with normal levels of IgG in serum. These data are given in Table 2.

We believe that clinicians should be aware of this potential interaction, especially since it is easily corrected. From our findings, it is reasonable to recommend, for those patients with hypergammaglobulinemia or those receiving high doses of intravenous IgG, that the daily dose of ceftriaxone be given in divided doses every 12 h instead of once every 24 h. This dosing regimen should minimize the duration of exposure of these patients to subinhibitory antibiotic concentrations in serum.

Finally, we only studied ceftriaxone, a drug with relatively insignificant toxicity even at high doses. There is a possibility that other drugs may interact similarly with gamma globulins. If such drugs have narrow therapeutic indices, the clinical relevance of this phenomenon would be greater.

REFERENCES

1. **Albengres, E.** 1986. Drug-immunoglobulin interactions, p. 95–101. *In* Protein binding and drug transport. Schattauer Verlag, Stuttgart, Germany.
2. **Arvidsson, A., G. Alvan, B. Angelin, O. Borga, and C. E. Nord.** 1982. Ceftriaxone: renal and biliary excretion and effect on the colon microflora. J. Antimicrob. Chemother. **10**:207–215.
3. **Balant, L., P. Dayer, and R. Auckenthaler.** 1985. Clinical pharmacokinetics of the third generation cephalosporins. Clin. Pharmacokinet. **10**:101–143.

4. **Balant, L., and J. Fabre.** 1979. Clinical relevance of different electrophoretic methods for the analysis of urinary proteins, p. 216–231. *In* U. G. Dubach and U. Schmidt (ed.), Diagnostic significance of enzymes and proteins in urine. Huber, Bern, Switzerland.
5. **Berkman, S. A., M. L. Lee, and R. P. Gale.** 1990. Review: clinical uses of intravenous immunoglobulin. Ann. Intern. Med. **112**:278–292.
6. **Bernard, E. P.** 1973. A calculation for finding binding parameters from a Scatchard plot. Anal. Biochem. **56**:306–309.
7. **Cantu, T. G., J. D. Dick, D. E. Elliott, R. L. Humphrey, and D. M. Kornhauser.** 1990. Protein binding of vancomycin in a patient with immunoglobulin A myeloma. Antimicrob. Agents Chemother. **34**:1459–1461.
8. **Hannah, E. R.** 1967. A graphical method for the determination and presentation of binding parameters in a complex system. Anal. Biochem. **20**:525–532.
9. **Helwig, J. T., and K. A. Council (ed.).** 1985. SAS user's guide: statistics version, 5th ed. SAS Institute Inc., Cary, N.C.
10. **Kim, S. K.** 1989. High-dose intravenous immune globulin impairs antibacterial activity of antibiotics. J. Allergy Clin. Immunol. **84**:579–588.
11. **Lueeke, R. H., and W. D. Wosilait.** 1986. Estimation of drug binding parameters. J. Pharm. Biopharm. **14**:65–78.
12. **McNamara, P. J., M. Gibaldi, and K. Stoeckel.** 1983. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding. I. Theoretical considerations. Eur. J. Clin. Pharmacol. **25**:399–405.
13. **McNamara, P. J., M. Gibaldi, and K. Stoeckel.** 1983. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding. II. Physiological significance. Eur. J. Clin. Pharmacol. **25**:407–412.
14. **McNamara, P. J., K. Stoeckel, and W. H. Ziegler.** 1982. Pharmacokinetics of ceftriaxone following intravenous administration of a 3 gram dose. Eur. J. Clin. Pharmacol. **22**:71–75.
15. **Newland, A. C.** 1986. Intravenous immunoglobulin therapy in chronic idiopathic thrombocytopenic purpura, p. 203–216. *In* A. Morell and U. E. Nydegger (ed.), Clinical use of intravenous immunoglobulins. Academic Press, Inc., London.
16. **Ochs, H. D., S. H. Fischer, R. J. Wedgwood, D. W. Wara, M. J. Cowan, A. J. Ammann, A. Saxon, M. D. Budinger, R. U. Allred, and R. H. Rousell.** 1984. Comparison of high-dose and low-dose intravenous immunoglobulin therapy in patients with primary immunodeficiency diseases. Am. J. Med. **76**:78–82.
17. **Pasko, M. T., and W. J. Jusko.** 1989. Affecting ceftriaxone plasma protein binding during heart surgery. J. Pharm. Sci. **78**:807–811.
18. **Patel, I. H., S. Chen, M. Parsonnet, M. R. Hackman, M. A. Brooks, J. Konikoff, and S. A. Kaplan.** 1981. Pharmacokinetics of ceftriaxone in humans. Antimicrob. Agents Chemother. **20**:634–641.
19. **Pirofsky, B.** 1984. Intravenous immune globulin therapy in hypogammaglobulinemia. A review. Am. J. Med. **76**:53–60.
20. **Priore, R. L., and H. E. Rosenthal.** 1976. A statistical method for the estimation of binding parameters in a complex system. Anal. Biochem. **70**:231–240.
21. **Rabat, E. A.** 1976. Structural concepts in immunology and immunochemistry, 2nd ed. Holt, Rinehart and Winston, New York.
22. **Schaad, U. B., L. M. William, and S. Klaus.** 1984. Single-dose ceftriaxone kinetics in the newborn. Clin. Pharmacol. Ther. **37**:522–528.
23. **Sophianopoulos, J. A., S. J. Durham, A. J. Sophianopoulos, H. L. Ragsdale, and W. P. Cropper.** 1978. Ultrafiltration is theoretically equivalent to equilibrium dialysis but much simpler to carry out. Arch. Biochem. Biophys. **178**:132–137.
24. **Stoeckel, K.** 1981. Pharmacokinetics of Rocephin, a highly active new cephalosporin with an exceptionally long biological half-life. Chemotherapy **27**(Suppl. 1):42–46.
25. **Stoeckel, K.** 1983. Pharmacokinetics of ceftriaxone, a long-acting broad-spectrum cephalosporin. *In* Progress in therapy of bacterial infection. A new cephalosporin: ceftriaxone. APCS no. 19. Excerpta Medica, Amsterdam.
26. **Stoeckel, K., P. McNamara, R. Brandt, H. Plozza-Nottebrock, and W. Ziegler.** 1981. Effects of concentration-dependent plasma protein binding of ceftriaxone kinetics. Clin. Pharmacol. Ther. **26**:650–657.
27. **Stoeckel, K., H. Tuerk, V. Trueb, and P. J. McNamara.** 1984. Single-dose ceftriaxone kinetics in liver insufficiency. Clin. Pharmacol. Ther. **36**:500–509.
28. **Wagner, J. G.** 1975. Fundamentals of clinical pharmacokinetics, p. 28. Drug Intelligence Publications Inc., Hamilton, Ill.
29. **Walter, C.** 1974. Graphical procedure for the detection of deviation from the classical model of enzyme kinetics. J. Biol. Chem. **249**:699–703.
30. **Whitlam, J. B., and K. F. Brown.** 1981. Ultrafiltration in serum protein binding determinations. J. Pharm. Sci. **70**:146–150.
31. **Winston, D. J., R. B. Pollard, and W. G. Ho.** 1987. Intravenous immune globulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. Ann. Intern. Med. **106**:12–18.