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Serum Protein-Binding Characteristics of Vancomycin

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A synthesis of studies of serum protein binding of vancomycin and its reported abnormal binding in serum with very high concentrations of immunoglobulin A (IgA) suggests that this antibiotic may be bound to more than one serum protein. Using an ultrafiltration method for separating free from bound drug and high-performance liquid chromatography to measure drug concentration, we studied the binding characteristics of vancomycin for α-1 acid glycoprotein, IgG, IgM, IgA, and albumin. The results showed that vancomycin does not bind to α-1 acid glycoprotein, IgG, or IgM. Major binding to albumin and IgA occurs, and total drug binding to serum proteins can be fully explained by binding to these two proteins. We calculate an *N* (number of binding sites per molecule) of 1.3 ± 0.4 and a *K* (association constant) of $3.3 \times 10^5 \pm 6.3 \times 10^4$ M^{-1} (*NK* = 4.3×10^5 M^{-1}) for binding to IgA, whereas the corresponding *NK* value for albumin was only 527.5 M^{-1}, indicating that vancomycin preferentially binds to IgA. Very high concentrations of IgA in serum (i.e., grams per deciliter), such as in patients with IgA myeloma, may result in the paradox of high (total) concentrations of vancomycin in serum that may be clinically ineffective.

Vancomycin has been reported to be from 10 to 82% (mean, 55%) protein bound in pooled serum (1, 3, 4, 9–12, 15, 16, 19). These reports provide no detailed studies of binding to specific serum proteins, and it is not clear which protein or proteins are the major binding proteins in serum. In fact, information regarding specific binding to albumin is not adequate. The large variations noted (10 to 82%) could be due to a number of factors that include variations between subjects, differences in drug and/or protein concentrations, or differences in assay methods. More important, however, since these studies did not report or control albumin concentrations in the samples, is that these variations could be due to multiple binding proteins in serum. In two studies, it was shown that as albumin concentration in serum increased, the free fraction of vancomycin decreased (4, 15), but in another study in which albumin concentration was measured, the authors found no correlation between albumin concentration and free drug (1). Recently, Cantu et al. reported a case of atypical vancomycin pharmacokinetics observed in a patient with immunoglobulin A (IgA) myeloma with serum IgA concentrations varying from 3,600 to 4,610 mg/dl (3). The patient's serum albumin, total protein, and IgM concentrations were within the normal range, but the IgG concentration was below normal. The free drug in this patient was only 3% of the total, while in control patients free drug concentrations were in the range of 62 to 90%. These results strongly suggest that vancomycin binds to more than one serum protein and that IgA may be an important vancomycin-binding protein in human serum. In view of these, we studied binding characteristics of vancomycin for various serum proteins.

MATERIALS AND METHODS

Pooled normal serum was obtained from normal volunteers. Serum samples with various concentrations of IgA or IgM were collected from patients with IgA or IgM myeloma. α-1 acid glycoprotein and human serum albumin (HSA) were obtained from Sigma Chemical Company (St. Louis, Mo.), and IgG solution was obtained from Cutter Biological, Elkhart, Ind. Vancomycin standard and vancomycin for infusion were from Eli Lilly & Co. (Indianapolis, Ind.).

Preliminary experiments indicate that vancomycin does not bind to the CENTRIFREE micropartition system filter membrane (Amicon Division, W. R. Grace & Co., Beverly, Mass.) regardless of whether phosphate-buffered saline (PBS) or protein-free normal serum ultrafiltrate is used as the solvent medium. Therefore, we used the ultrafiltration method for separating free from bound drug (17). Samples were placed in the filter and equilibrated at 37°C for 30 min and then centrifuged at 1,500 × *g* for 20 min at the same temperature. The free drug concentration in the ultrafiltrate was then quantitated. Vancomycin concentrations in protein solution, ultrafiltrate, and serum were determined by the ion-pair, reversed-phase high-performance liquid chromatography method (8, 13). The standard curve was linear from 0.1 to 100 μg/ml. The limit of detection was 0.1 μg/ml, at which the within-day coefficient of variation was less than 4% and the overall day-to-day coefficient of variation was less than 7% in either protein solutions, serum ultrafiltrate, or whole serum.

In other preliminary experiments, we found that vancomycin was 70% bound to purified HSA when PBS was used as a solvent medium and 25 to 30% bound to purified HSA when normal serum ultrafiltrate was used. Consequently, normal serum ultrafiltrate was used as the medium in subsequent experiments.

Specific immunoglobulins were quantitated by a nephelometric procedure (N Immunoglobulin kits, Behring Diagnostics, Inc., Somerville, N.J.), and albumin was quantitated by the Kodak Ektachem Clinical Chemistry Slide Test (Eastman Kodak Co., Rochester, N.Y.).

In vitro studies of drug binding. The binding kinetics of vancomycin for IgG or HSA were determined by adding the drug into IgG (20 to 50 g/liter) or HSA (2 to 4 g/dl) solutions.

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The vancomycin-IgA binding kinetics were determined by adding the drug into various IgA myeloma serum samples for which IgA (0 to 3,000 mg/dl), IgG (200 to 529 mg/dl), IgM (13 to 3,040 mg/dl), and albumin (2,220 to 4,000 mg/dl) concentrations were known. Binding was studied at various concentrations of vancomycin (0.1 to 100 μg/ml).

In vivo studies. The in vivo protein binding of vancomycin in a patient with IgA myeloma was studied. The patient was administered a single intravenous dose of vancomycin. Venous blood samples (4 ml) were obtained at time zero and at various times after dosing. Unfortunately, we were able to obtain serum samples at only three time points from this patient. Sera were stored at −90°C. Determinations of free and protein-bound drug were carried out as described above.

Data analysis. Actual total (C_{total}) and free (C_{free}) drug concentrations were obtained from the experiments. Bound drug (C_{bound}) concentration was calculated from the equation $C_{bound} = C_{total} - C_{free}$. The drug protein binding parameters were estimated from the following equation (2, 5, 12, 18):

$$C_{total} = C_{free} + C_{bound} \quad (1)$$

$$= C_{free} + \sum_{\substack{i=1 \\ j=1}}^{\substack{i=n \\ j=m}} \frac{N_{i,j} \times P_j \times K_{i,j} C_{free}}{1 + K_{i,j} \times C_{free}}$$

where C_{free} and C_{bound} are the molar free drug concentration and molar bound drug concentration, respectively; P_j is the molar concentration of a specific jth binding protein; m is the number of types of binding proteins; n is the number of classes of binding sites on the specific jth binding protein; and $N_{i,j}$ and $K_{i,j}$ are the number of binding sites and the corresponding association constant for the ith class of binding site on the jth binding protein, respectively. The equation was fitted to the experimental data with a nonlinear least-squares regression model with SAS PROC NLIN (7), and all initial estimates were determined from Scatchard plots (6, 14). A model-discriminating F-ratio test and the difference (residual) between measured and computer-fitted concentrations of drug were used to determine the appropriate model (2, 5).

Prediction of serum vancomycin free concentration. From equation 1, it is difficult to calculate C_{free} from a known C_{total} when more than one binding protein and/or more than one binding site exists. We therefore took a series of theoretical C_{free} values and calculated their corresponding C_{total} by using known protein binding parameters. C_{free} was then plotted as a function of C_{total}, and specific C_{free} values were then derived from the plot.

RESULTS

Vancomycin did not bind to IgG even at high IgG concentrations of up to 50 g/liter. Vancomycin also did not bind to 200 mg of α-1 acid glycoprotein per liter. Changes in IgM concentration from 0 to 3,040 mg/dl (with HSA and IgA concentrations remaining relatively constant) did not alter the percent C_{bound} vancomycin (Table 1), which suggests that vancomycin does not substantially bind to IgM. In view of these findings, the concentrations of IgG, IgM, and α-1 acid glycoprotein were ignored in subsequent experiments.

Binding of vancomycin to HSA was drug concentration independent. About 25% of vancomycin was bound at an HSA concentration of 3 to 4 g/dl. Plotting C_{bound} versus C_{free} yielded the binding site and association constant product (NK) of 527.5 M^{-1} (Fig. 1).

TABLE 1. Percent C_{bound} vancomycin in the presence of increasing IgM concentrations[a]

Concn of protein in serum:			% C_{bound} vancomycin
HSA (g/dl)	IgA (mg/ml)	IgM (mg/dl)	
4.0[b]	0	0	30
3.0	67	13	25
3.1	56	3,040	23

[a] C_{total} of vancomycin, ≥50 μg/ml.
[b] This sample is HSA dissolved in ultrafiltered normal human serum.

The percentages of C_{bound} vancomycin in serum samples with various concentrations of HSA and IgA are shown in Fig. 2 and 3. No correlation between HSA concentration and percent C_{bound} in sera was observed when calculations were uncorrected for various concentrations of IgA (Fig. 2a). A correlation between percent C_{bound} vancomycin and IgA concentration was observed, one which improved as the C_{total} increased, i.e., r = 0.29, 0.59, and ca. 0.77 at C_{total} values of 10, 50, and 80 μg/ml, respectively (Fig. 2b). The correlation improved further after correction for the contribution of HSA binding (r = 0.34, 0.64, and 0.87 at C_{total} values of 10, 50, and 80 μg/ml, respectively). C_{bound} varied directly with IgA concentration and indirectly with C_{total} drug concentration except at very low IgA concentrations (0.01 g/dl) where C_{bound} did not change as a function of C_{total} (Fig. 3).

The Scatchard plot of data obtained from various serum samples indicates that there are at least two binding sites or binding proteins for vancomycin in serum. A representative plot is shown in Fig. 4. A model assigning IgA and albumin as the binding proteins resulted in a good fit (Fig. 4 insert). The IgA binding parameters estimated from the data for the 11 patients with the model were $N = 1.3 \pm 0.4$ and $K = 3.3 \times 10^5 \pm 6.3 \times 10^4$ M^{-1}. These results are consistent with the view that IgA and serum albumin are the major binding proteins in serum for vancomycin.

On the basis of the above data showing that percent C_{bound} is linear and independent of C_{total} when C_{total} is above 50 μg/ml, a simplified mathematical model can be generated to

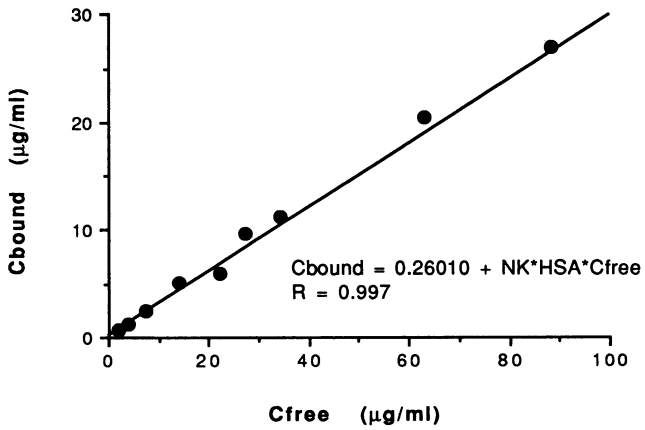

FIG. 1. Plot of C_{bound} versus C_{free}. Where HSA = $5.88 \cdot 10^{-4}$ M, the linear regression line was $C_{bound} = NK \cdot HSA \cdot C_{free}$ and NK = 527.5 M^{-1}.

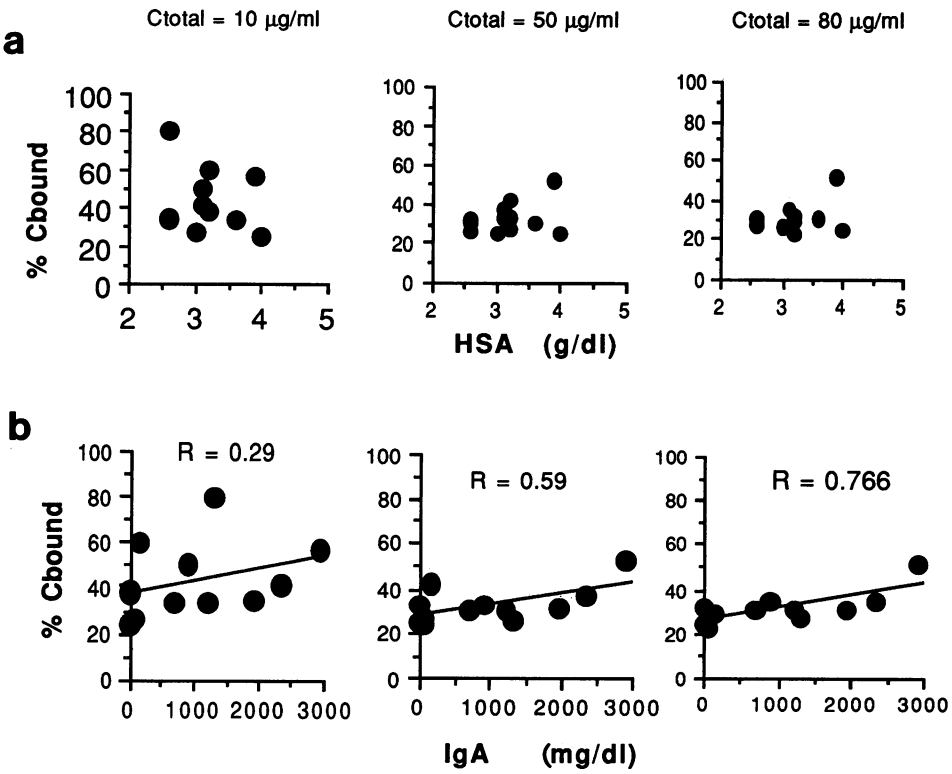

FIG. 2. The percent C_{bound} vancomycin in the presence of serum with various concentrations of albumin and IgA. (a) No correlation between albumin concentration and percent C_{bound} vancomycin was observed. (b) A correlation between IgA concentration and percent C_{bound} vancomycin was observed, one which improved as the vancomycin concentration increased.

predict percent C_{bound} when C_{total} is greater than 50 μg/ml:

$$\% C_{bound} = 6.45 \cdot [\text{HSA (g/dl)} + \text{IgA (g/dl)} + 1] \quad (2)$$

$$\% C_{free} = 100 - \{6.45 \cdot [\text{HSA (g/dl)} + \text{IgA (g/dl)} + 1]\} \quad (3)$$

With equation 2, the predicted percent C_{bound} correlated with the observed value (Fig. 5, $r = 0.84$). Note that for C_{total} below 50 μg/ml, equation 1 should be used because percent

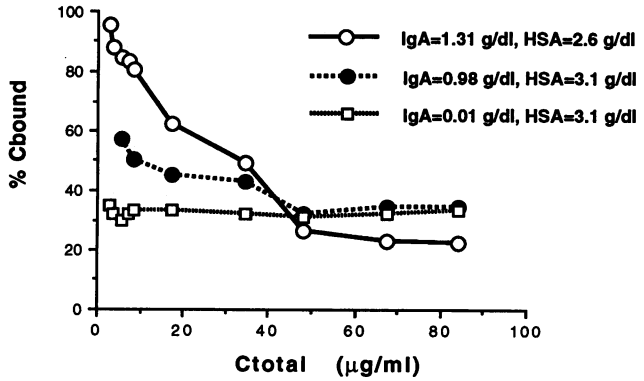

FIG. 3. Protein binding of vancomycin to serum with various concentrations of IgA and relatively constant concentrations of albumin. As IgA concentration increased, the C_{bound} increased nonlinearly except at very low IgA concentrations of 0.01 g/dl, at which it was essentially linear with a mean percent C_{bound} value of ~30%.

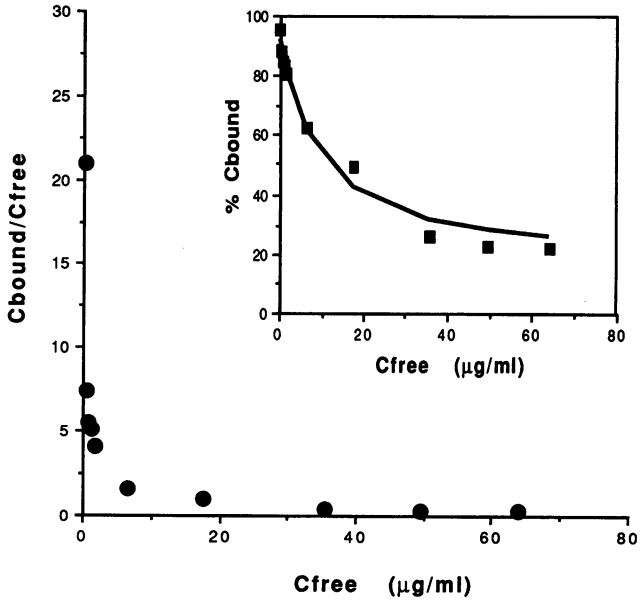

FIG. 4. The Scatchard plot of vancomycin protein binding kinetics obtained for patients with higher concentrations of IgA. The results suggest the presence of at least two binding sites or binding proteins in the serum and are consistent with binding to IgA and albumin. The inset shows results of fitting vancomycin serum protein binding data with equation 1. A model consisting of two binding proteins in which one was albumin fitted the data very well. IgA protein binding parameters were, therefore, estimated by nonlinear regression analysis.

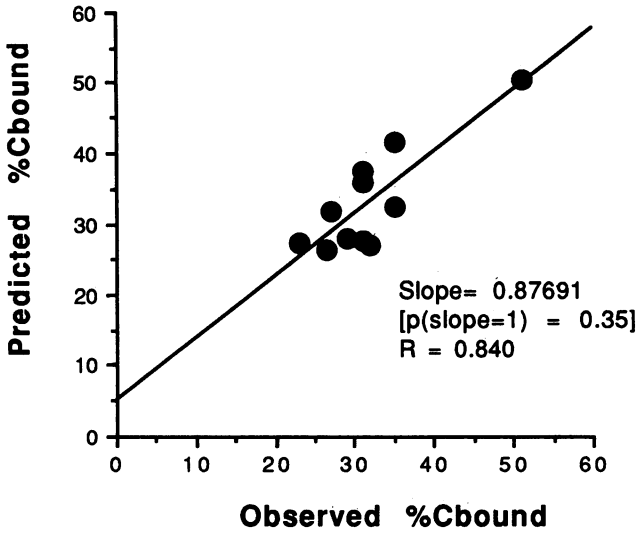

FIG. 5. Plot of predicted versus observed percent C_{bound} vancomycin in IgA myeloma serum with a total vancomycin concentration of greater than 50 μg/ml. The prediction was made by using equation 2.

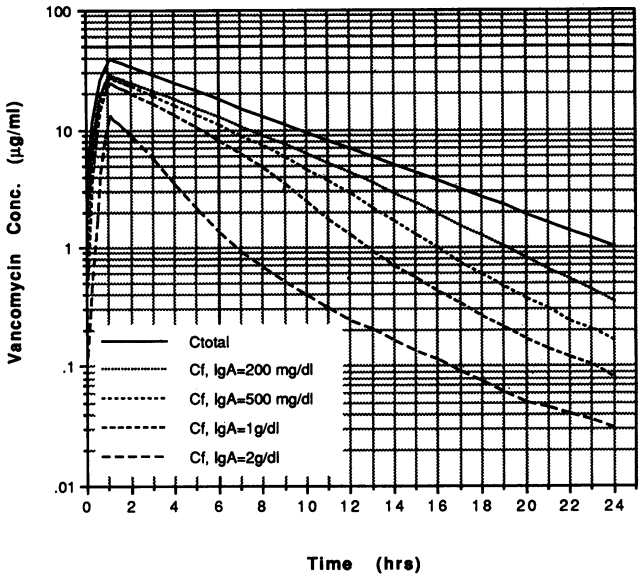

FIG. 6. Simulated total and free vancomycin concentration-time profile for a 70-kg man who is administered 1 g of vancomycin given intravenously over 1 h. An albumin concentration of 4 g/dl and IgA concentrations of 200, 500, 1,000 and 2,000 mg/dl were calculated to illustrate the effect of IgA on free vancomycin concentration. C_{total}, total vancomycin concentration; C_f, free or effective vancomycin concentration. Pharmacokinetic parameters used were elimination rate constant = 0.16 h^{-1} and volume of distribution = 25.6 liters, one-compartment model. Calculation did not take into account changes in the elimination rate and the volume of distribution as protein binding increases.

C_{bound} varied indirectly with C_{total} and the relationship was not linear as shown in Fig. 3.

In the in vivo study, measured vancomycin free fractions were compared with the predicted values. The predicted and observed values are also in good agreement (Table 2). Pharmacokinetic parameters, however, cannot be calculated because we do not have enough datum points for this patient.

DISCUSSION

Our findings indicate that vancomycin binds primarily to both IgA and albumin and that it does not bind to IgM, IgG, and α-1 acid glycoprotein. Serum protein binding increased as the concentrations of IgA and albumin increased, and it decreased (nonlinearly) as the vancomycin concentration increased except at very low IgA concentrations at which bound drug stayed at approximately 30% regardless of vancomycin concentration. Although it is possible that vancomycin binds to additional proteins in serum, binding to IgA and albumin is sufficient to explain most if not all total serum protein binding, and additional binding to other normal serum proteins would be insignificant.

What is the significance of our data? We believe that our findings adequately explain the large variations in serum protein binding of vancomycin reported in the literature. Our data also confirm the clinical observations of Cantu et al. (3) who reported that at serum IgA concentrations in grams per deciliter, the pharmacokinetics of vancomycin may be adversely affected and, if not considered, may lead to a surprising paradox of very high serum drug levels and a dismal clinical outcome. From our data, we can estimate that, given a normal serum albumin concentration, a serum IgA concentration exceeding 1,000 mg/dl will significantly shorten the time above the MIC of free vancomycin. The calculated serum concentration-time profile for total and free vancomycin for a hypothetical 70-kg man who is given 1,000 mg of vancomycin intravenously and whose serum albumin concentration is 4 g/dl and serum IgA concentration is 200 to 2,000 mg/dl is shown in Fig. 6. Table 3 gives the calculated free concentration values at various total vancomycin and IgA concentrations in a patient whose serum albumin concentration is 4 g/dl. This figure and this table can be used to estimate the potential effect of high serum IgA on the effective (free) concentration of vancomycin. For patients

TABLE 2. Comparison of measured and predicted free and bound vancomycin[a]

C_{total} (μg/ml)	C_{free} (μg/ml) (% C_{bound})	
	Observed	Predicted
<0.1	<0.1	
5.5	2.2 (60)	2.16 (58)
10.25	4.84 (52.8)	4.76 (49)

[a] For a patient who received a 1-g intravenous dose of vancomycin. Serum albumin and IgA concentrations were 4.4 g/dl and 736 mg/dl, respectively.

TABLE 3. Theoretical free vancomycin concentrations (C_{free}) at various total vancomycin (C_{total}) and serum IgA concentrations[a]

C_{total} (μg/ml)	C_{free} at serum IgA concn (mg/dl):				
	250	500	1,000	2,000	3,000
0.1	0.05	0.02	0.01	0.00	0.00
1.0	0.35	0.16	0.08	0.04	0.01
5	2.83	1.64	0.69	0.16	0.05
10	7.35	5.73	3.36	0.50	0.07

[a] Serum albumin concentration was set at 4 g/dl.

with IgA concentrations exceeding 1,200 mg/dl, we suggest the use of another antibiotic. If vancomycin is still to be used, determination of free drug concentration is necessary.

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