Serum Protein Binding of Prednisolone in Four Species

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Received August 10, 1979, from the *Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo and the Millard Fillmore Hospital, Buffalo, NY 14209.* Accepted for publication March 6, 1980.

Abstract □ The protein binding of prednisolone in serum from humans, rabbits, dogs, and rats was determined by equilibrium dialysis. Two nonlinear least-squares regression programs were used to characterize transcortin and albumin binding of the steroid. The animal species differed markedly in their ability to bind prednisolone, but the rabbit was most similar to humans in transcortin concentration and affinity constant.

Keyphrases □ Prednisolone—serum protein binding in humans, rabbits, dogs, and rats □ Serum protein binding—prednisolone in humans, rabbits, dogs, and rats □ Binding, serum protein—prednisolone in humans, rabbits, dogs, and rats

The serum protein binding of prednisolone is nonlinear in humans due to saturation of the high affinity binding sites on the α_1-glycoprotein, transcortin (1). As serum concentrations of the steroid increase, the limited capacity of transcortin for binding is exceeded and the fraction of free drug in serum increases. This nonlinear serum protein binding is partially responsible for the dose-dependent disposition of prednisolone observed in humans (2). To examine its pharmacokinetics further, an animal model was sought that would exhibit protein binding characteristics for prednisolone similar to those observed in humans.

EXPERIMENTAL

The serum protein binding of prednisolone was examined in Sprague–Dawley rats, New Zealand White rabbits, a mongrel dog, and a normal man. Serum was spiked with prednisolone in concentrations ranging from 0.024 to 25 μM. The tritiated prednisolone was purified by high-pressure liquid chromatography (HPLC). A 10-μl aliquot (10,000 dpm) of [^{3}H]prednisolone (specific activity of 53 Ci/mmole) was added to 0.8 ml of the serum samples. The samples then were dialyzed against an equal volume of isotonic phosphate buffer (pH 7.4) using plexiglass cells maintained in a water bath at 37°. After dialysis, the counts per minute of the buffer and serum samples were converted to disintegrations per minute using the sample channel ratio technique. The percent of prednisolone bound and the molar concentrations of free (D_F) and bound (D_b) drug then were calculated for each serum sample.

The relationship that governs the protein binding of prednisolone is:

$$D_b = \frac{N_T K_T P_T D_F}{1 + K_T D_F} + \frac{N_A K_A P_A D_F}{1 + K_A D_F} \qquad \text{(Eq. 1)}$$

where N_T and N_A are the number of binding sites on the transcortin and albumin molecules, K_T and K_A are the affinity constants for transcortin and albumin, and P_T and P_A are the molar serum concentrations of transcortin and albumin (3). Albumin binding apparently was nonspecific; *i.e.*, the product of K_A and D_F for the albumin–prednisolone interaction was negligible when compared to unity, permitting simplification of Eq. 1 to yield:

$$D_b = \frac{N_T K_T P_T D_F}{1 + K_T D_F} + N_A K_A P_A D_F \qquad \text{(Eq. 2)}$$

The nonlinear least-squares regression computer program, NONLIN (4), was employed to fit the binding data to Eq. 2. The D_b and D_F variables for each serum sample served as the dependent and independent variables. Least-squares estimates of K_T, $N_T K_T P_T$, and $N_A K_A P_A$ then were computed. The N_T and N_A values were assumed to be equal to one. Albumin concentration (P_A) was measured chemically[1] in all serum samples, making solution of Eq. 2 for all binding parameters possible. The three computer-generated binding parameters (P_T, K_T, and K_A) are apparent values since endogenous corticosteroids present in the serum are likely to compete with prednisolone for protein binding sites.

Least-squares estimates of K_T, $N_T K_T P_T$, and $N_A K_A P_A$ also were obtained by the statistical method of Priore and Rosenthal (5). This method employs the total drug concentration and the fraction bound as the independent and dependent variables. The use of these variables minimizes the effects of experimental error on the magnitude of the generated binding parameters through the use of the most error-free independent variable (total drug concentration). An even distribution of the experimentally determined values around the fitted fraction bound *versus* the total drug concentration binding curve is produced.

[1] Technicon autoanalyzer.

RESULTS AND DISCUSSION

Figure 1 illustrates the protein binding profile for each species as a function of the postdialysis serum prednisolone concentration. All profiles exhibited nonlinearity consistent with saturation in transcortin binding and linear binding to albumin. Dog and rat serum demonstrated the least concentration dependence in the protein binding of prednisolone. The therapeutic range of the serum prednisolone concentration extended up to ~7 μM.

The binding parameters obtained using the modified NONLIN computer program (Method I) and using the MACMOL program of Priore and Rosenthal (Method II) are presented in Table I. The two methods yielded binding parameters in the same general range. There was excellent agreement for all binding parameters in the rabbit and in the K_A values obtained for each species. Differences between the methods existed in the P_T and K_T values obtained in the dog, rats, and human. At low serum prednisolone concentrations, where the protein binding of the steroid exhibits concentration dependence, small errors in the determination of either the fraction bound or the total drug concentration can introduce rather large errors in the calculated values of D_f (total drug concentration × free fraction). Such errors will affect most profoundly the binding parameters obtained using Method I, where D_f is the independent variable. Since this problem is avoided using Method II, it probably generates the closer estimates of the true binding parameters for transcortin.

At high prednisolone concentrations, where albumin is the major binding protein, the values of the prednisolone free fraction are larger and do not vary appreciably in any one species. As a result, the errors incurred in quantitation of the binding systems at high serum concen-

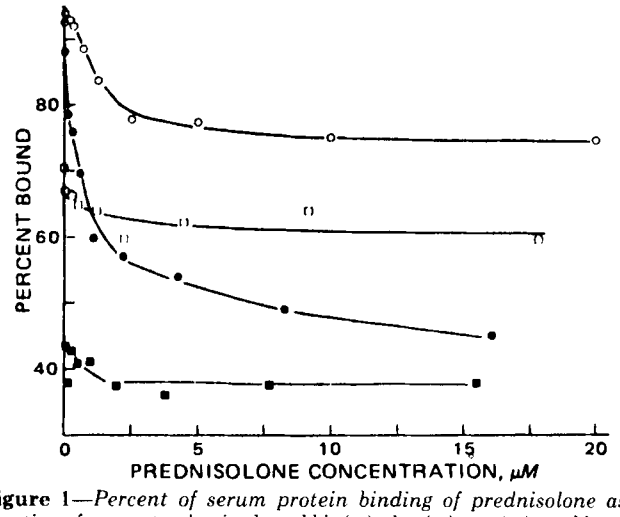

Figure 1—*Percent of serum protein binding of prednisolone as a function of concentration in the rabbit* (○), *dog* (□), *rat* (■), *and human* (●).

0022-3549/80/0800-0977$01.00/0

Table I—Serum Protein Binding Parameters for Prednisolone in Four Species Obtained by Methods I and II [a]

Species	Transcortin				Albumin		
	$P_T, M \times 10^7$		$K_T, M^{-1} \times 10^{-7}$		P_A [b], $M \times 10^4$	$K_A, M^{-1} \times 10^{-3}$	
	I	II	I	II		I	II
Rabbit	5.74	6.13	2.33	2.09	4.49	6.22	6.15
Dog	2.21	1.19	0.284	0.569	5.73	2.62	2.69
Rat	0.259	1.01	0.653	0.152	3.96	1.51	1.45
Human	6.19	3.26	0.499	1.63	6.43	1.21	1.400

[a] Method I is from Ref. 4, and Method II is from Ref. 5. [b] Determined by an automatic analyzer.

trations are of less significance, accounting for the good agreement in the K_A values between the two methods.

The binding profile of the rabbit appears most like humans in the degree of nonlinearity in prednisolone binding (Fig. 1). The binding constants in Table I (Method II) confirm this observation. The P_T and K_T values in the rabbit are closest to those values determined in humans, producing similar decreases in binding as the serum prednisolone concentration increases. The binding parameters confirm the presence of high affinity, low capacity transcortin binding and low affinity, high capacity albumin binding in each species.

The transcortin concentrations and affinity constants for all of the species examined are in the general range of values reported by other investigators for cortisol. Westphal (6) obtained P_T and K_T values in the rabbit of 3.6×10^{-7} M and 4×10^7 M^{-1}, which agree well with the present estimates. In contrast, the P_T and K_T values determined by Westphal in the rat were 6.9×10^{-7} M and 1×10^7 M^{-1}. These discrepancies may reflect inherent differences in the interactions of prednisolone and hydrocortisone with transcortin. The use of different strains of the same animal species and physiological factors such as age, sex, and endogenous corticosteroid level might affect the transcortin–steroid interaction and contribute to variability in the binding parameters (7).

In summary, the animal species studied differed markedly in their ability to bind prednisolone. The rabbit was the most similar to humans in transcortin concentration and affinity constant for prednisolone and in the range of free drug fractions at increasing steroid concentrations.

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ACKNOWLEDGMENTS

Supported in part by Grant 24211 from the National Institutes of General Medical Sciences.

The MACMOL computer program (5) was kindly provided by Dr. Roger L. Priore.

Effect of Sodium Bicarbonate on *In Vitro* Conversion of Fibrinogen to Fibrin

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Received November 8, 1979, from the *Division of Nuclear Medicine, Department of Radiology, Martin Luther King Jr. General Hospital and Charles R. Drew Postgraduate Medical School, Los Angeles, CA 90059.* Accepted for publication March 12, 1980.

Abstract □ The effect of sodium bicarbonate on the conversion of fibrinogen to fibrin clot was investigated using fresh human whole blood and pure human fibrinogen. *In vitro* experimental data demonstrated that sodium bicarbonate interfered with fibrin clot formation during clotting. This study assessed the possibility of synergistic action of sodium bicarbonate with sodium citrate. As expected, sodium citrate potentiated the anticoagulant action of sodium bicarbonate.

Keyphrases □ Sodium bicarbonate—effect on conversion of fibrinogen to fibrin, synergistic action with sodium citrate □ Sodium citrate—effect on fibrin clot formation, synergistic action with sodium bicarbonate □ Fibrin—effect of sodium bicarbonate and sodium citrate on *in vitro* conversion of fibrinogen

In vitro experimental results and clinical patient data demonstrate that sodium and potassium bicarbonate can interfere with the clotting process, as evidenced by prolongation in prothrombin time and thrombin clotting time (1). The anticoagulant effect appears to be caused primarily by the specific anion species and an increase in ionic strength and to a lesser extent by the effect of pH. Thrombin clotting time analyses on citrated whole blood samples containing sodium bicarbonate indicate that the unavailability of calcium ions is not the direct cause of clot inhibition.

Since previous work contained no information dealing directly with the effect of bicarbonate on clotting (2–9), the present study was undertaken to determine the effect of sodium bicarbonate on the conversion of pure human fibrinogen to fibrin clot. The possibility of synergistic action of sodium bicarbonate with sodium citrate on clot inhibition also was investigated.

EXPERIMENTAL

Lyophilized human fibrinogen[1] was reconstituted with water for injection USP to a concentration of 20 mg/ml at pH 6. Prior to the addition of fibrinogen, a predetermined amount of sodium bicarbonate solution[2] (pH 7.8, 7.5%), ranging from 1 to 25 mg in a volume of <0.1 ml, was added to each test tube containing 3.0 ml of 7 mM pH 7.4 Sorensen's phosphate buffer (ionic strength 0.017) and 0.4 ml of a 3.8% sodium citrate solution (pH 9.85, 0.13 M, ionic strength 0.078). The resulting mixture contained one part of citrate solution to nine parts of test sample. The amount of citrate solution used was equivalent to the quantity of anticoagulant present in the blood-collecting test tube. When the required amount of sodium bicarbonate solution to be added to the test samples exceeded 0.1 ml, a weighed amount of reagent grade powder[3] was dissolved in the

[1] Parenogen, Cutter Laboratories, Berkeley, Calif.
[2] Abbott Laboratories, North Chicago, Ill.
[3] American Drug and Chemical Corp., Los Angeles, Calif.

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