PLASMA PREDNISOLONE CONCENTRATIONS: COMPARISON OF RADIOIMMUNOASSAY AND COMPETITIVE PROTEIN BINDING ASSAY

A. V. Tembo, J. W. Ayres, E. Sakmar, M. R. Hallmark and J. G. Wagner
College of Pharmacy and Upjohn Center for Clinical Pharmacology
The University of Michigan
Ann Arbor, Michigan 48109

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ABSTRACT

A comparison was made between plasma concentrations of prednisolone measured by both competitive protein binding radioassay (CPB) and radioimmunoassay (RIA) and, with each assay, using a calibration curve generated from individual subject data and from pooling the individual calibration curve data. The plasma samples were obtained from six normal adult male volunteers who were pretreated with dexamethasone to suppress endogenous hydrocortisone and who then ingested 10 mg of prednisolone. Both the standard curve data and the plasma concentrations were evaluated statistically. It was shown that the CPB method has considerably greater precision than the RIA method and could be employed in bioavailability and pharmacokinetic studies of both prednisolone and prednisone. It was also shown that corticosteroid binding globulin cross-reacts considerably less with the major metabolite of prednisolone, 20β-dihydroprednisolone, than the particular antiserum used in the RIA.

INTRODUCTION

Following single small oral doses of either prednisolone (11β,17, 21-trihydroxy-1,4-pregnadiene-3,20-dione) or prednisone (17α,21-dihydroxypregna-1,4-diene-3,11,20-trione), plasma concentrations of prednisolone have been measured by radioimmunoassay (RIA) [1-9] or by competitive protein binding (CPB) [10-15]. Both analytical procedures are extremely sensitive, require only small plasma volumes and are rapid and simple to perform. However, both corticosteroid binding globulin utilized in the CPB method, and antisera to prednisolone so far developed and utilized in the RIA method, cross-react with several endogenous and synthetic steroids [1,9,16,17]. The RIA method requires the use of a special antiserum, which is expensive to produce and is not readily available to most laboratories. Also, the precision of the RIA method with antisera prepared in two different laboratories is relatively poor, with coefficients of variation ranging from 12-20% for one antiserum and from 20-26% with the other antiserum, when these were calculated from concentrations inversely estimated from the standard curves [7].

Since the CPB radioassay utilizes only diluted blank (pre-dose) plasma and no antiserum, it would appear to be the preferred method of assay. This article compares results obtained when the two methods were applied to the same 60 plasma samples.

In the application of both CPB and RIA methods to plasma samples containing unknown amounts of the compound being assayed there are basically two methods, both of which are widely used. In the one method (individual subject calibration method) the blank (pre-dose) plasma or serum is spiked with known amounts of the steroid being measured and these spiked samples are assayed at the same time as the "unknown" plasma samples; the concentrations of the "unknowns" are estimated only from the calibration data for that subject. In the other method (pooled calibration method) the standard curve is prepared from the pooled data obtained by spiking some or all of the subjects' zero hour plasma. Although there is considerable literature on the mathematics and statistics pertaining to the RIA and CPB methods [19-32] a comparison of plasma concentrations of prednisolone estimated using both of these methods with either the CPB or RIA assay methods does not appear to have been reported. In this report plasma concentrations of prednisolone were estimated by both of the calibration methods using % bound values obtained by both the RIA and CPB methods.

Although the prednisolone antiserum used to measure the plasma concentrations in the study reported has been utilized in normal volunteers without suppression of endogenous hydrocortisone with dexamethasone [3,6] we chose to use dexamethasone in this study as in others [2,4,5,7,8] in order to make the assays more specific since both corticosteroid binding globulin and the prednisolone antiserum [1] react with hydrocortisone and do not react with dexamethasone [1,16,17]. The CPB method would most probably be useful also in patients receiving high doses of either prednisone or prednisolone since such patients would have very low plasma concentrations of endogenous cortisol. It should be noted that corticosteroid binding globulin binds prednisone much less than prednisolone, and only about 10 ng of prednisone can be detected by the CPB assay, whereas about 1 ng of prednisolone can be detected.

EXPERIMENTAL

Study Protocol. Study conditions and blood sampling were the same as previously described [7] and plasma samples from six subjects were utilized for the current report.

Assay of Plasma Samples. For the RIA method antiserum was kindly supplied by W. A. Colburn, who has characterized it [1]. Plasma samples were assayed by the modification by Sullivan et al. [2] of the radioimmunoassay of Colburn and Buller [1]. Extraction of prednisolone by the dichloromethane from diluted plasma was shown to be quantitative. Blank (pre-dose) plasma and "unknown" plasma samples were diluted 1:5, 1:10 or 1:25 with water and the degree of dilution was shown not to affect the results. For calibration purposes solutions containing 0, 2, 4, 6, 10 and 20 ng of unlabeled prednisolone per ml of diluted (1:25) plasma were prepared from each of the six subjects' blank plasma. Both these solutions and diluted "unknown" plasma samples were assayed in duplicate independently. The calibration solution and "unknowns" for a given subject were assayed on the same day, but different days were involved with the six different subjects. Hence the pooled calibration plots include both intra- and inter-day variation.

For the CPB method solutions containing 0, 2, 4, 6 and 10 ng of unlabeled prednisolone were prepared in the same diluted (1:25) blank plasmas as utilized in the RIA. "Unknown" plasma samples were usually diluted 1:25, but some had to be diluted 1:5 to bring them into the region of the standard curve. One ml aliquots of diluted plasma were extracted with 7 ml of dichloromethane for 5 min on a mechanical shaker. Samples were centrifuged for 5 min, the aqueous phase was aspirated off, and 5 ml aliquots of the organic phase were transferred to culture tubes and the dichloromethane evaporated under a gentle flow of nitrogen.

To each dried residue in the culture tube was added 10 µl (3700 cpm) of tritiated prednisolone solution followed by 1 ml of phosphate buffered saline solution (pH = 7.4). One ml of freshly prepared corticosteroid binding globulin solution was added. The corticosteroid binding globulin solution was prepared by adding 0.3 ml of blank plasma to a 25 ml volumetric flask and making up to volume with water. All water used in both the RIA and CPB assays had a resistance of about 18 megohm and was obtained from a millipore Super Q system. Unused corticosteroid binding globulin solution from each subject was discarded. The samples (above) were mixed thoroughly and allowed to incubate for 20 min in a 45°C shaking water bath. After 20 min, the samples were placed in an ice bath and incubated for an additional 30 min. At the end of the incubation period, 0.5 ml of pre-cooled (3°C) dextran-coated charcoal (2.5% charcoal and 0.25% dextran T_{70}) was added. The samples were allowed contact with the charcoal for 10 min, and were then centrifuged at 0°C for 10 min. One ml of supernatant was pipetted out of each culture tube and placed in a numbered scintillation vial containing 10 ml of Unogel ®. All samples were counted for 10 min in a Packard Model 3320 Tri-Carb Liquid Scintillation Spectrometer. All assays were run in duplicate as in the RIA procedure.

The cross-reactivity studies with the prednisolone metabolite, 20β-dihydroprednisolone (11β,17,20β,21-tetrahydroxy-1,4-pregnadien-3-one), were carried out in phosphate buffer without extraction for both RIA and CPB.

Calibration data for both the RIA and CPB methods were used to determine the parameters ln Q (intercept) and slope (s) of a logarithmic-logistic equation, namely,

$$\ln \left[\frac{100 - y}{y}\right] = \ln Q + s \cdot \ln C \qquad \text{Eq. 1}$$

where $y = \frac{B(X)}{B(0)} \times 100$, B(X) is the mean (of duplicates) % bound at prednisolone concentration, C, and B(0) is the mean (of duplicates) % bound in the absence of prednisolone. In this type of plot the slope is positive, whereas Rodbard *et al.* [18] and others chose to plot $\ln \left[\frac{y}{100 - y}\right]$ on the ordinate, which provides a straight line with the same, but negative slope.

Table 1

Cross-Reactivity of the Prednisolone Metabolite, 20β-Dihydroprednisolone, with the Prednisolone Antisera (RIA) and Corticosteroid Binding Globulin (CPB)

	% Bound					
	RIA				CPB	
	Batch 1[a]		Batch 2[b]			
Conc.(ng/ml)	M[c]	P[d]	M	P	M	P
0	37.5[e]	37.5[e]	---	19.9[f]	49.3	49.0
			---	19.9[f]	49.3	50.3
2	24.2	33.4	20.6	7.79	48.5	35.9
			19.7	7.95	47.5	37.1
4	22.4	29.3	16.6	4.58	47.5	24.8
			17.1	5.05	45.8	26.2
6			15.5	3.39	46.7	18.0
			16.1	3.62	45.8	17.9
10	19.3	18.8	13.3	2.49	44.9	10.9
			13.8	2.65	43.4	10.5
20		7.4	11.3	1.19	41.4	8.00
			11.1	1.01	42.6	8.26

[a]Batch 1 of the prednisolone antiserum was used in previous studies.

[b]Batch 2 of the prednisolone antiserum was used in the study described in this article.

[c]M = Metabolite.

[d]P = Prednisolone.

[e]Averages of duplicate assays in both columns (the individual % bound values were inadvertently destroyed).

[f]Duplicate independent assays performed on the same day.

RESULTS AND DISCUSSION

Table 1 lists cross-reactivity data for the metabolite in both the RIA and CPB methods. The low B(0) values for prednisolone with batch 2 of the prednisolone antiserum (Table 1) is the result of using only 20 µl of antiserum in the particular experiment which measured cross-reactivity

with the metabolite and is not representative of either those B(0) values observed with batch 1 or in the calibration data (compare Table 2) where 30 µl of antiserum were used. Data in Table 1 clearly indicate that corticosteroid binding globulin in the CPB assay cross-reacts with the principal prednisolone metabolite, 20β-dihydroprednisolone, to a lesser degree than prednisolone antiserum in the RIA assay.

Table 2

Calibration Data for Prednisolone Assays Using a 1:25 Dilution of Zero Hour Plasmas[a] and Coefficients of Variation Calculated from Duplicate Assays

		% Bound at Prednisolone Plasma Conc. of							
Assay	Subject	0	2	4	6	10	20 ng/ml	Mean	C.V.(%)
RIA	1	36.2[b]	19.9	14.3	10.7	5.89	2.98	14.5	7.81
		33.3[b]	20.5	13.0	8.51	5.61	2.86		
	2	30.5	20.5	14.3	11.0	7.65	4.63	14.8	3.36
		31.7	19.3	14.3	11.0	7.40	4.75		
	3	30.9	19.3	14.1	12.5	7.65	4.70	15.1	3.24
		31.3	20.8	14.4	12.5	8.24	4.60		
	4	29.2	17.7	11.0	7.87	5.16	3.85	12.7	12.5
		34.4	16.1	10.3	7.95	5.56	3.44		
	5	27.5	18.1	12.0	9.48	6.59	4.48	13.1	3.52
		27.9	18.5	12.9	9.49	6.40	3.31		
	6	34.1	21.7	13.3	9.62	6.61	3.98	14.8	3.15
		34.5	20.2	12.9	9.68	6.52	4.10	overall	
	Mean	31.8	19.3	13.1	10.0	6.61	3.97	Mean 14.1	
	C.V.(%)[c]	5.55	4.47	3.98	6.31	3.64	9.16	C.V.(%) 6.28	
CPB	1	47.4	37.9	29.5	22.2	13.7	---	30.6	2.37
		48.7	38.7	31.1	22.2	14.3	---		
	2	38.1	29.9	22.1	18.7	11.7	---	24.4	4.13
		38.9	29.9	25.0	17.7	12.0	----		
	3	45.3	34.6	27.8	21.5	14.1	---	28.5	4.06
		45.8	31.3	28.1	20.7	15.3	---		
	4	47.9	37.2	29.8	22.3	14.3	---	30.4	1.39
		48.1	36.9	30.5	21.7	15.2	---		
	5	44.0	33.8	26.0	20.7	13.9	---	27.6	2.14
		44.1	33.5	26.9	19.1	13.8	---		
	6	52.4	40.4	30.4	24.4	16.7	---	32.6	2.94
		49.5	39.9	30.6	25.1	16.8	---	overall	
	Mean	45.9	35.3	28.2	21.4	14.3		Mean 29.0	
	C.V.(%)	2.09	2.82	3.61	3.03	3.36		C.V.(%) 2.90	

[a] Pre-dose plasma after dexamethasone suppression of endogenous hydrocortisone.

[b] Duplicate independent assays performed at the same time.

[c] C.V.(%) = $\frac{\text{S.D.}}{\text{Mean}} \times 100$ where S.D. = $\sqrt{\frac{\Sigma d^2}{2N}}$ where d = difference between duplicates and N = number of pairs of duplicates.

Table 2 lists the primary calibration data for both the RIA and CPB methods with the means and coefficients of variation estimated from duplicate % bound values using the formula of Cekan [30]. The CPB has considerably greater precision than the RIA method as reflected by the appreciably lower coefficients of variation.

Table 3 lists the ranges, means, standard deviations and coefficients of variation of the response variable (i.e., the ordinates of the logistic-logarithmic calibration plot). Greater precision is again observed for the CPB assay. Similar statistics have been utilized formerly [25,32,33,34]. Severe nonuniformity of variance, as reported by Rodbard et al. [32], was not observed with either method of assay.

Table 3

Range, Mean, Standard Deviation and Coefficient of Variation of Parameter on the Ordinate of the Pooled Logistic-Logarithmic Calibration Plots. $\ln\left[\left\{100 - \frac{B(X)}{B(0)} \times 100\right\} \Big/ \left\{\frac{B(X)}{B(0)} \times 100\right\}\right]$ corresponding to indicated concentration of prednisolone

Assay	Parameter	2	4	6	10	20 ng/ml
RIA	Range[a]	-0.1242 to 0.6678	0.1603 to 0.4853	0.6411 to 1.1040	1.0669 to 1.6143	to 1.7268 2.3879
	Mean[a]	-0.4565	0.3551	0.8211	1.3399	1.9547
	S.D.	0.2029	0.2127	0.2052	0.2405	0.2542
	C.V.(%)[b]	20.5	21.5	20.7	24.4	25.8
CPB	Range	-0.9579 to 1.3683	-0.4043 to -0.5348	0.0568 to 0.1914	0.7163 to 0.8886	
	Mean	-1.2143	-0.4649	0.1392	0.7914	
	S.D.	0.1434	0.0568	0.0492	0.0608	
	C.V.(%)	14.4	5.68	4.93	6.08	

[a] Ranges and mean for six subjects' pre-dose plasmas with added prednisolone.

[b] C.V.(%) = $100\sqrt{e^{\sigma^2} - 1}$ where σ is the standard deviation calculated from the natural logarithms.

Table 4 gives the intercepts, slopes and correlation coefficients of the logistic-logarithmic equation (equation 1) obtained from both individual subject and pooled calibration data obtained by RIA and CPB methods. These indicate that the slopes of the logarithmic-logistic calibration plots for individual subjects are reasonably homogenous with the coefficients of variation of the slopes being 8.08 and 8.79% for the RIA and CPB, respectively. However, the intercepts (ln Q values) for individual subject data are less homogenous for the RIA than for the CPB

with the coefficients being 20.9 and 8.79%, respectively. An indication of relative sensitivity may be obtained by two criteria from the pooled calibration parameters in Table 4. The concentration corresponding to 50% response, i.e., $[y = \frac{B(X)}{B(0)} \times 100 = 50]$, EC_{50}, is equal to $Q^{-1/s}$ [34]; for the RIA method EC_{50} = 2.9 ng/ml and for the CPB method EC_{50} = 2.57 ng/ml. Also, from equation 1, when the concentration, C, is equal to 1 ng/ml, ln C = 0 and Q = (100 - y)/y whence y = 100/(1 + Q); for the RIA method the calculated value of y is 75.5% and for the CPB method the corresponding value was 84.0%, corresponding to C = 1 ng/ml. These values suggest that the CPB assay was just slightly more sensitive than the RIA. The sensitivity of an assay, as pointed out by Cekan [31], in the sense of distinguishing one concentration from another is also clearly dependent on precision. Both the precision and the sensitivity of the CPB method are greater than that of the RIA.

Table 4

Intercepts, Slopes and Correlation Coefficients of Individual Subject (I) and Pooled (P) Logistic-Logarithmic Calibration Plots of $\ln\left[\left\{100 - \frac{B(X)}{B(0)} \times 100\right\} \Big/ \left\{\frac{B(X)}{B(0)} \times 100\right\}\right]$ versus ln C where C is Concentration of Prednisolone (ng/ml).

	RIA			CPB		
Subject	Intercept	Slope	Correlation Coefficient	Intercept	Slope	Correlation Coefficient
1	-1.1757	1.1939	0.9995	-2.3958	1.4144	0.998
2	-1.2306	1.0085	0.998	-2.1703	1.2797	0.999
3	-1.2458	1.0070	0.9995	-1.7883	1.0730	0.989
4	-0.6764	0.9462	0.993	-2.1796	1.2883	0.995
5	-1.3338	1.0730	0.997	-2.0456	1.2268	0.999
6	-1.0729	1.0705	0.994	-2.1755	1.2562	0.9997
Ave.	-1.1225	1.0499		-2.1259	1.2564	
C.V.(%)[a]	20.9	8.08		9.42	8.79	
From Pooled Data	-1.1260	1.0501	0.970	-2.1263	1.2564	0.992

[a] $C.V.(\%) = \frac{\text{standard deviation}}{|\text{mean}|} \times 100.$

Table 5 provides the means, coefficients of variation and bias obtained from inversely estimated concentrations using the calibration data for both assay methods. Because of the nature of data collection these coefficients of variation include both intra- and inter-day

variation and in the view of the authors represent the best indication of error in the assay methods employed. Both the bias and coefficients of variation are smaller for the CPB than the RIA assay.

Table 5

Means, Coefficients of Variation and Bias of Inversely Estimated Concentrations of Prednisolone (C^a) from Logistic-Logarithmic Equations Obtained with Pooled Data.

Assay Method			Actual Concentration of Prednisolone (ng/ml)				
			2	4	6	10	20
RIA		Mean	1.92	4.17	6.49	10.7	19.3
		C.V.(%)	20.5	21.0	19.8	22.9	26.3
	Bias	ng/ml	-0.8	+0.17	+0.49	+0.7	-0.7
		%	-4.2	+4.2	+7.6	+6.5	-3.6
CPB		Mean	2.07	3.75	6.06	10.2	
		C.V.(%)	11.7	4.37	3.92	4.85	
	Bias	ng/ml	+0.07	-0.25	+0.06	+0.02	
		%	+3.4	-6.7	+1.0	+2.0	

a- $C = e^{\left[\ln\left\{\frac{100 - y}{y}\right\} - \ln Q\right] / s}$ where $y = \frac{B(X)}{B(0)} \times 100$. Each mean and C.V. is based on the six values of y (one for each subject) used to prepare the standard curve. For the RIA assay: ln Q = -1.1260 and s = 1.0501; for the CPB assay: ln Q = -2.1263 and s = 1.2564.

Table 6 lists the plasma concentrations of prednisolone estimated by all four methods--i.e., by both RIA and CPB methods and each using individual subject (I) and pooled (P) calibration data. An attempt was made to determine which of the four methods gives the "more correct" answers. Table 7 gives the statistics of the least squares regression lines when the plasma concentration measured by RIA assay was plotted against the plasma concentration measured by CPB assay for the 0.25-12 hr time range given in Table 6. The 24 hr concentrations of Table 6 were excluded since the 24 hr concentrations measured by the CPB method were appreciably higher than those measured by the RIA method in 5 out of the six subjects by both calibration methods. Also, for subjects 1-5 in the 2-12 hr time range and subject 6 in the 4-12 hr time range each set of plasma concentrations listed in Table 6 are log-linear on semilogarithmic graph paper, but the 24 hr plasma concentration is almost always higher than that predicted by extrapolating the trend lines. These facts, plus similar observations from previous studies [2,4,5,7,8] strongly suggest that there is some recovery of

Table 6. Prednisolone Plasma Concentrations

Subj.	Assay Method	Calibration Method	Prednisolone Plasma Concentration (ng/ml) at Indicated Sampling Time (hrs)									
			0.25	0.5	1	2	3	4	6	8	12	24 hr
1	RIA	I[a]	8.79	64.9	170.0	203.0	298.0	219.0	126.0	77.7	27.9	1.89
		P[b]	8.21[c]	78.2[c]	231.0[c]	283.0[c]	296.0[c]	220.0[c]	165.0[c]	95.8[c]	30.1[c]	1.45[c]
	CPB	I	11.0	70.1[d]	156.0[d]	237.0[d]	195.0[d]	160.0[d]	87.9[d]	51.6[d]	27.0	15.4
		P	9.67[e]	63.9[d]	158.0[d]	254.0[d]	203.0[d]	162.0[d]	82.4[d]	45.1[d]	26.9[e]	14.2[e]
2	RIA	I	64.0	192.0	223.0	229.0	159.0	123.0	73.9	36.2	10.2	3.57
		P	52.8[c]	150.0[c]	173.0[c]	178.0[c]	125.0[c]	98.4[c]	60.5[c]	30.7[c]	9.21[c]	3.38[c]
	CPB	I	56.7	201.0	220.0	194.0	132.0	91.9	45.5[d]	35.6	10.1	96.0
		P	55.2[d]	202.0[d]	221.0[d]	195.0[d]	131.0[d]	90.5[d]	44.1[d]	35.6[e]	9.80[e]	98.1[e]
3	RIA	I	32.0	81.9	159.0	234.0	198.0	130.0	78.4	34.5	8.29	3.17
		P	26.8[e]	65.6[c]	123.0[c]	178.0[c]	152.0[c]	102.0[c]	63.0[c]	28.8[c]	9.88[c]	2.98[c]
	CPB	I	29.6	45.6	117.0	190.0	144.0	87.3	49.7	30.2	8.29	102.0
		P	29.8[d]	54.3[d]	122.0[d]	184.0[d]	146.0[d]	94.8[d]	58.4[d]	30.3[e]	10.0[e]	86.3[e]
4	RIA	I	45.1	46.6	193.0	218.0	165.0	138.0	56.0	19.3	4.53	0.91
		P	45.1[e]	64.5[d]	237.0[d]	263.0[d]	206.0[d]	175.0[d]	78.2[d]	30.2[d]	7.49[d]	1.79[c]
	CPB	I	58.8	110.0	193.0	204.0	166.0	108.0	55.7	40.8	16.0	95.8
		P	60.0[d]	109.0[d]	195.0[d]	207.0[d]	167.0[d]	107.0[d]	54.1[d]	41.7[e]	15.7[e]	98.3[e]
5	RIA	I	189.0	240.0	231.0	212.0	165.0	123.0	58.7	17.2	8.10	1.58
		P	159.0[d]	202.0[d]	194.0[d]	178.0[d]	138.0[d]	102.0[d]	48.6[d]	14.0[d]	6.60[c]	1.27
	CPB	I	163.0	221.0	184.0	166.0	118.0	94.8	66.9	29.5	8.23	56.9
		P	166.0	223.0	187.0	167.0	121.0	97.6	69.2	30.1[e]	8.60[e]	57.3[e]
6	RIA	I	14.1	94.1	275.0	259.0	220.0	168.0	118.0	72.0	34.6	7.58
		P	14.7[d]	99.9[d]	296.0[d]	278.0[d]	236.0[d]	180.0[d]	125.0[d]	76.2[d]	36.4[d]	7.83[d]
	CPB	I	11.7	94.7	256.0	204.0	196.0	117.0	89.5	62.6	43.5	2.54
		P	11.2[e]	90.6[d]	244.0[d]	196.0[d]	188.0[d]	112.0[d]	85.6[d]	59.8[d]	41.5[d]	2.40[d]

a - I = Individual subject calibration plot used.
b - P = Pooled calibration plot used, based on data for all six subjects.
c - Plasma diluted 1:10.
d - Plasma diluted 1:25.
e - Plasma diluted 1:5.

hydrocortisone levels in the 12-24 hr period. The greater reactivity of corticosteroid binding globulin with hydrocortisone than the prednisolone antiserum with hydrocortisone most probably explains the trend in most of the 24 hr plasma concentrations.

From the data in Table 7 it may be seen that none of the intercepts of the least squares lines were significantly different from zero ($p \geq .05$) when the plasma concentration measured by RIA was plotted against the plasma concentration measured by CPB and the slopes of the least squares lines forced through the origin are also listed in the last column of the table. For the individual subject data two of the slopes of the lines were significantly different from unity ($p \leq .05$), but the non-unity slope occurred for different subjects when individual subject calibration data were used than when pooled calibration data were used. Table 7 shows that plasma levels predicted using pooled CPB standard curves bear about the same relationship to plasma levels obtained from pooled RIA standard curves as do plasma levels from individual CPB standard curves when compared to plasma levels obtained from individual RIA standard curves.

Table 8 lists both the arithmetic and geometric means and the corresponding coefficients of variation, calculated from the concentrations and the natural logarithms of the concentrations, respectively, using the plasma concentrations measured by RIA and CPB methods. These data, along with Table 7, suggest that the individual subject calibration method offers no real advantage over the pooled calibration method and supports the similar conclusion made before [7].

The final problem is to determine whether the RIA or CPB is giving the "more correct" answer.

Figure 1 is a plot of the difference between the plasma concentration measured by RIA and the plasma concentration measured by CPB against the average of the plasma concentration measured by the two methods, when pooled calibration parameters (Table 4) were employed. This type of plot is taken from Neter and Wassermer [35]. Only the plasma concentrations in the 0.25-12 hr time range were utilized in preparation of the plot. It can be seen that the RIA method gives lower plasma concentrations than the CPB method for low concentrations

Table 7. Statistics of Least Squares Regression Lines When RIA Assay Plotted Versus CPB Assay Using Plasma Concentrations in 0.25 to 12 Hour Range (N = 9 for Individual Subjects and N = 54 for Pooled Plot).

Calibration Method	Subj.	Line Free to Pass Through Any Intercept: Intercept[a]	Standard Error of Intercept	Slope	Standard Error of Slope	Correlation Coefficient	Slope of Line Forced Through the Origin
Individual Subject	1	9.19	25.2	1.12	0.189	0.913	1.17
	2	13.2	10.4	1.00	0.078	0.979	1.09
	3	6.78	7.18	1.28[b]	0.074	0.988	1.33
	4	-19.1	16.9	1.10	0.137	0.951	0.928
	5	-4.92	12.0	1.23[c]	0.089	0.982	1.19
	6	2.21	12.2	1.15	0.086	0.981	1.16
	Pooled Data	3.08	6.39	1.12	0.050	0.953	1.14
Pooled Data	1	21.2	18.3	1.21	0.133	0.960	1.34
	2	14.4	8.41	0.760[b]	0.0632	0.977	0.849
	3	2.90	3.44	0.991	0.0349	0.996	1.02
	4	-17.7	20.7	1.32	0.166	0.949	1.20
	5	-6.09	9.85	1.03	0.0717	0.983	0.987
	6	0.95	12.8	1.30[d]	0.0942	0.982	1.30
	Pooled Data	1.99	7.86	1.11	0.0611	0.930	1.12

[a] All intercepts are not significantly different from zero ($p \geq .05$).

[b,c,d] Slopes are significantly different from unity at b, $.01 > p > .001$; c, $.05 > p > .02$; d, $.02 > p > .01$. All other slopes are not significantly different from unity.

Table 8

Means and Coefficients of Variation Calculated from Prednisolone Plasma Concentrations Measured by RIA and CPB Methods in the Time Range 0.25 - 12 hr

Calibration Method	Parameter	1	2	3	4	5	6	Overall
Individual Subject	Arithmetic Mean[a]	122.	117.	92.1	102.	128.	129.	115.
	C.V.(%)[a]	25.7	12.6	26.4	17.7	17.1	15.8	19.5
	Geometric Mean[b]	83.4	82.6	63.3	70.0	86.5	94.1	79.3
	C.V.(%)	19.4	15.0	23.6	42.8	18.9	14.8	24.1
Pooled Data	Arithmetic Mean	134.	103.	82.1	115.	117.	132.	114.
	C.V.(%)	29.3	17.2	4.84	24.4	8.18	24.2	22.0
	Geometric Mean	88.3	74.1	59.8	80.3	79.8	94.9	78.7
	C.V.(%)	29.7	12.8	5.95	29.7	21.6	20.5	21.7

[a]Assumes a normal distribution and C.V.(%) = $\frac{\text{S.D.}}{\text{Mean}} \times 100$ where S.D. = $\sqrt{\frac{\Sigma d^2}{2N}}$ where d = difference between concentrations measured by RIA and CPB methods and N = number of pairs (N = 9 for individual subjects and N = 54 for overall values).

[b]Assumes a log-normal distribution and C.V.(%) = $100\sqrt{e^{\sigma^2} - 1}$ where σ is the S.D. calculated the same way except d = difference in the natural logarithms of concentrations measured by RIA and CPB methods.

of drug, and higher plasma concentrations than the CPB method for higher concentrations of drug. These differences are a reflection of the bias of the standard curves for each analytical method as presented in Table 5 and plotted in Figure 2. Since the overall bias of the CPB method is lower, the precision greater, the cross reactivity with the major metabolite of prednisolone lower, and the procedure more readily available for general laboratory usage it is clear that the CPB method is more desirable than the RIA method. It is therefore concluded that the CPB method using a standard curve generated from pooling daily standard curves is more desirable than the RIA when measuring plasma concentrations of prednisolone, when the patient's or subject's plasma is cortisol-free or essentially so.

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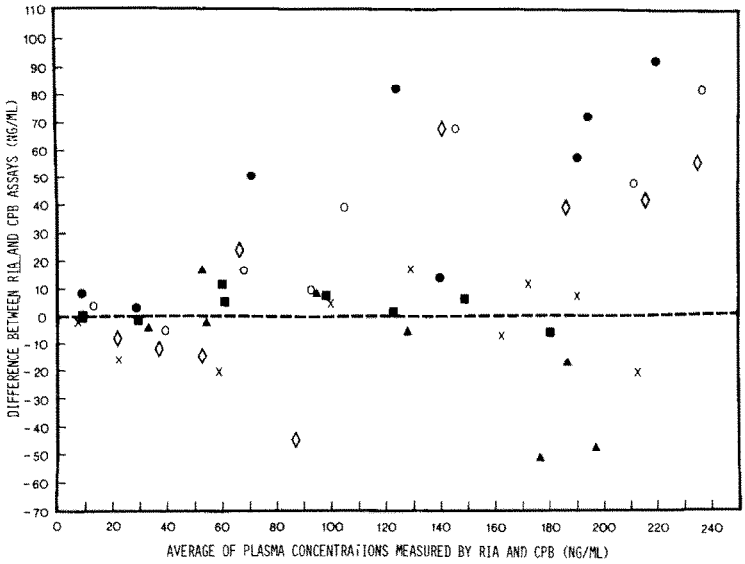

Fig. 1. A plot of difference between the plasma prednisolone concentration measured by RIA and the plasma concentration measured by the CPB method vs. the average of the two plasma concentrations.
Key: Subject 1, ●; 2, ▲; 3, ■; 4, X; 5, ◊; 6, O.

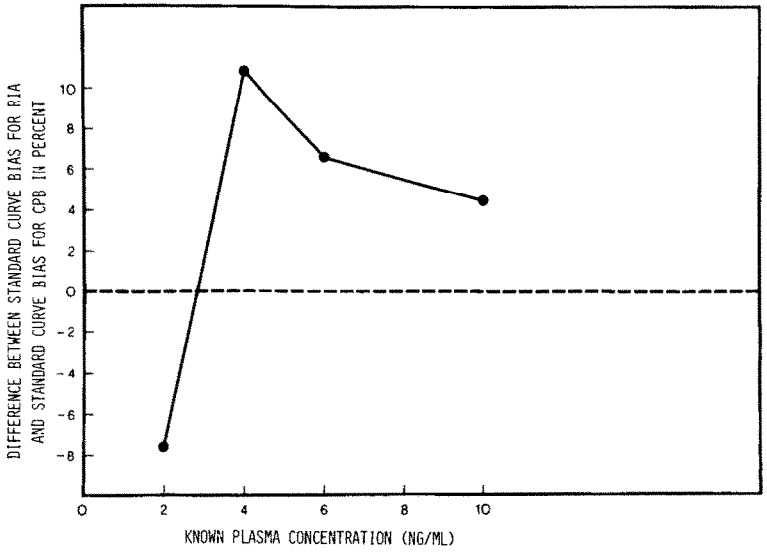

Fig. 2. A plot of the difference between the pooled standard curve bias for prednisolone concentration as measured by RIA and CPB vs. the known concentrations.

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Send reprint requests to: Dr. John G. Wagner, Upjohn Center for Clinical Pharmacology, The University of Michigan, Ann Arbor, Michigan, 48109.