In Vitro and *In Vivo* Availability of Commercial Prednisone Tablets

TIMOTHY J. SULLIVAN, ERMELINDA SAKMAR, KENNETH S. ALBERT, DONALD C. BLAIR, and JOHN G. WAGNER ˣ

Abstract □ A three-way crossover bioavailability study was performed using nine adult male volunteers with three different commercial prednisone tablets. Plasma samples were assayed for prednisolone, the active metabolite of prednisone, by a radioimmunoassay method. Statistical analysis showed significant differences in the rate of appearance of prednisolone in plasma but not in the amount converted to prednisolone. The results suggest that differences in *in vivo* rates of appearance of prednisolone in plasma correlate with *in vitro* rates of dissolution.

Keyphrases □ Prednisone—*in vitro* and *in vivo* bioavailability of commercial tablets □ Bioavailability—commercial prednisone tablets

Previous studies (1, 2) demonstrated that the clinical effectiveness of prednisone tablets can be correlated with *in vitro* dissolution rates. Two generic prednisone tablets that were inactive clinically dissolved much slower than a clinically active brand of prednisone tablet. Based at least in part on this discovery, USP XVIII included a dissolution test for prednisone tablets (3).

It is well documented that orally administered prednisone is rapidly metabolized to prednisolone, which is believed to be the bioactive form (4–6). With the development of a radioimmunoassay for prednisolone (6), it is now possible to measure the plasma levels of prednisolone following oral administration of single, low doses of prednisone.

This paper reports the results of a three-way crossover bioavailability study with commercially available prednisone tablets.

EXPERIMENTAL

The *in vitro* dissolution studies and content uniformity tests were performed in the laboratories of the Food and Drug Administration (FDA) according to USP XVIII specifications.

A three-way crossover bioavailability study was performed using nine healthy adult male volunteers between the ages of 24 and 31 years and weighing between 67.6 and 93.4 kg. The criteria for subject selection were previously reported (7). In each phase of the crossover study, each subject ingested 10 mg of prednisone as two 5-mg tablets of Tablet A, B, or C[1]. The treatment schedule is shown in Table I. Treatments were separated by 1 week.

The evening before each treatment period, 1.0 mg of dexamethasone was administered orally to each subject to suppress endogenous secretion of cortisol. The suppression was maintained by administration of 0.5 mg of dexamethasone 8 hr after the administration of prednisone.

Whole blood samples were taken from a forearm vein at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hr postdosing with prednisone. Each sample was centrifuged as soon as possible after collection; the plasma was placed in a stoppered vial, quick-frozen, and kept frozen until just prior to assay.

[1] Tablet A was Meticorten, 5 mg (Schering Corp.), Lot No. 2ABB804; Tablet B was prednisone, 5 mg (Marshall Pharmacal Corp., South Hackensack, N.J.), Lot No. 7165; and Tablet C was prednisone, 5 mg (Heather Drug Co., Cherry Hill, N.J.), Lot No. 090156.

Table I—Treatment Schedule

Subjects	Group	Phase I (Week 1)	Phase II (Week 2)	Phase III (Week 3)
1, 2, 3	I	A	B	C
4, 5, 6	II	B	C	A
7, 8, 9	III	C	A	B

Table II—Results of USP Tests on Tablets Used in Human Study

Tablet	Content Uniformity, % of Label/Tablet		Dissolution Test, % Dissolved/20 min	
A	Range 91.3–100.7, average 96.8		110.8	116.4
			111.6	117.8
			115.0	114.3
			108.0	111.4
			108.8	111.4
			110.6	112.2
			Average	112.4[a]
			SD[b]	2.96
B	90.8	87.4	15.9[c]	10.5[c]
	92.3	84.8	12.2[c]	7.6[c]
	88.4	92.1	15.3[c]	12.4[c]
	121.7[c]	95.4		
	93.5	96.6	Average	12.3
			SD	3.08
	Average	94.2		
	SD	10.3		
C	99.7	105.5	8.9[c]	12.6[c]
	98.6	100.2	10.5[c]	14.0[c]
	101.9	100.0	13.0[c]	9.5[c]
	99.0	97.6		
	96.2	96.7	Average	11.4
			SD	2.07
	Average	99.6		
	SD	2.78		

[a] The higher than label values are most probably caused by nonsteroidal components which absorb at the wavelength used in the tablet dissolution test. [b] Standard deviation. [c] Not in compliance with USP specifications.

All plasma samples were assayed for prednisolone using a modification of the procedure of Colburn and Buller (6), described previously (7).

All calibration curves were fitted essentially perfectly to a biexponential equation using the program NONLIN and a computer[2]. The amount of drug in the experimental plasma samples was then determined with an iterative program using an electronic calculator[3].

RESULTS AND DISCUSSION

In Vitro—Results of the USP content uniformity and tablet dissolution tests are summarized in Table II. Tablet A passed both the content uniformity and the tablet dissolution tests as well as the assay test (101.0% of label). Tablets B and C failed the tablet dissolution test since they gave values of 12.3 and 11.4% dissolved, respectively; the USP requires at least 60% of the labeled amount of prednisone to be dissolved in 20 min.

[2] IBM 360/67.
[3] Hewlett-Packard 9100.

Table III—Results of Nine Subject Three-Way Crossover Prednisone Study of Prednisolone Plasma Concentrations

Hours	Average Plasma Concentrations, ng/ml[a] Tablet A	Tablet B	Tablet C	Significance Level[b]	Tukey's Multiple Comparison Test at $p = 0.05$ Level[c] Tablet A *versus* B	Tablet A *versus* C
0.25	76.9 (110)	5.67 (161)	9.89 (225)	$0.01 < p < 0.025$	Sig.	Sig.
0.50	198 (63.3)	27.7 (63.4)	69.1 (60.0)	$p < 0.001$	Sig.	Sig.
1	254 (31.4)	111 (30.2)	185 (41.2)	$0.001 < p < 0.005$	Sig.	N.S.
2	250 (16.0)	164 (21.3)	228 (12.3)	$p < 0.001$	Sig.	N.S.
3	202 (18.3)	184 (15.7)	240 (14.9)	$0.001 < p < 0.005$	N.S.	Sig.
4	154 (14.9)	178 (15.7)	190 (18.6)	$0.01 < p < 0.025$	N.S.	Sig.
6	83.2 (30.5)	103 (19.2)	105 (39.9)	N.S. $(0.10 < p < 0.25)$	N.S.	N.S.
8	39.6 (33.2)	48.3 (36.2)	48.6 (49.2)	N.S. $(p > 0.25)$	N.S.	N.S.
12	19.3 (30.6)	20.0 (59.2)	22.6 (61.0)	N.S. $(p > 0.25)$	N.S.	N.S.
24	3.46 (178)	10.7 (162)	4.23 (156)	N.S. $(p > 0.25)$	N.S.	N.S.

[a] Averages are followed by percent coefficient of variation (standard deviation/mean × 100) in parenthesis. [b] Based on treatment mean square from the analysis of variance for crossover design. [c] Sig. = significant, and N.S. = not significant.

Table IV—Summary of Results of Nine Subject Three-Way Crossover Prednisone Study—Some Pharmacokinetic Parameters

Parameter	Parameter Value[a] Tablet A	Tablet B	Tablet C	Significance Level[b]	Tukey's Multiple Comparison Test at $p = 0.05$ Level[c] Tablet A *versus* B	Tablet A *versus* C
Area 0→12 hr, ng/ml × hr	1290 (15.9)	1100 (15.4)	1320 (22.2)	N.S. $(0.05 < p < 0.10)$	N.S.	N.S.
ln (area 0→12)	7.15	6.99	7.16	N.S. $(0.10 < p < 0.20)$		
Area 0→24 hr, ng/ml × hr	1430 (16.7)	1290 (20.2)	1430 (21.5)	N.S. $(p > 0.25)$	N.S.	N.S.
Peak concentration[d], ng/ml	285 (19.4)	192 (15.1)	255 (12.9)	$p < 0.001$	Sig.	N.S.
Peak time, hr[d]	1.28	3.00	2.11	$p < 0.001$	Sig.	Sig.
Half-life, hr	2.58 (20.2)	2.43 (25.7)	2.38 (27.6)	N.S. $(p > 0.25)$	N.S.	N.S.
Area $(0\rightarrow\infty)$/ mg/kg dose × half-life	4190	3860	4620	N.S. $(p > 0.25)$		

[a,b,c] See footnotes to Table III. [d] From observed peak plasma levels of individual subjects.

Tablet B failed the initial phase of the content uniformity test since one of the 10 tablets tested was out of compliance, but the results of the second phase of this test were not available. Tablet C passed the content uniformity test. These results were of interest because all three lots of tablets were purchased on the open market by the FDA.

In Vivo—One lot of generic tablets (Tablet B or C) had a history of clinical failure, and the following is a statement written by the FDA concerning this lot (designated 078-692F by the FDA):

> *The FDA documented therapeutic failures with the prednisone tablets in two patients afflicted with congenital adrenal hyperplasia in December, 1971. Immediate follow-up by FDA's Newark District Office ascertained that the subject lot of tablets failed the USP dissolution test. Further investigation by the FDA revealed that the product was no longer in the channels of distribution and thus was not available for recall or seizure. A "Consent Decree of Permanent Injunction" was signed by the firm on May 1, 1972 in the U.S. District Court of New Jersey which stipulated that the firm will not manufacture and introduce products into interstate commerce which fail to meet appropriate standards.*

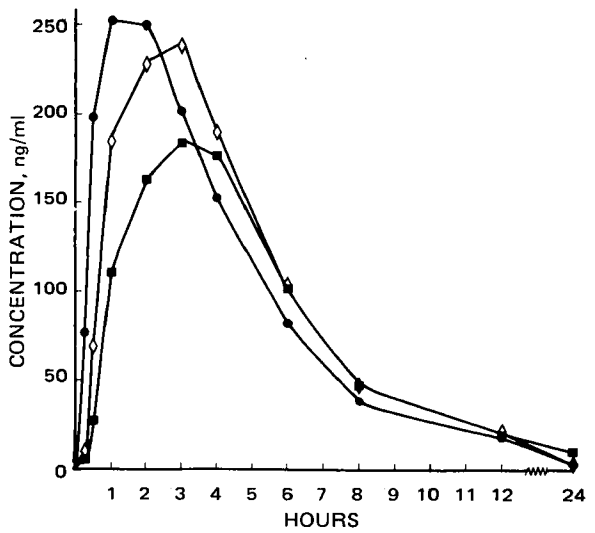

Figure 1—*Average plasma concentrations of prednisolone following oral administration of prednisone as Tablet A (●), Tablet B (■), or Tablet C (◊).*

In the human bioavailability study, it was important to consider the cross-reactivity of prednisolone and cortisol with the antiserum (7). To obtain a specific assay for prednisolone, it was necessary to suppress endogenous secretion of cortisol. This suppression was accomplished by the administration of dexamethasone (8). Any effect of dexamethasone on the conversion of prednisone to prednisolone is unknown, but it would be expected to be minimal and unimportant under the study conditions because of the time interval between doses and the relative magnitudes of the doses.

Dexamethasone, even in high concentrations, does not interfere with the binding of prednisolone to the antibody. Prednisone has also been shown not to interact significantly with the antibody (6).

Tables III and IV summarize the results of the bioavailability study and the statistical analysis of the data. It is apparent from Table III that there are significant differences in the rates of appearance of prednisolone in plasma. Analyses of variance of plas-

ma concentrations indicated highly significant differences among average plasma concentrations from 0.25 to 4 hr, inclusive. Tukey's Multiple Comparison Test (9) was used to identify significant differences between treatment pairs. The test indicated significant differences during the absorptive phase when Tablet A was compared to either Tablet B or C. The particular patterns of significant differences between treatments can be rationalized by considering the average plasma level curves for each (Fig. 1).

Differences in peak plasma concentrations and time of the peak were also seen (Table IV). The average peaks and rates of appearance were in the order: A > B or C. Since Tablet A was the innovator's product and considered the "standard" preparation, comparisons of only Tablet A *versus* B and Tablet A *versus* C were made. No statistical comparison of Tablet B *versus* C should be inferred (Tables III and IV).

After the 4-hr sampling time, the differences among average plasma prednisolone concentrations were not significant ($p > 0.10$). At the 24-hr sampling time, 11 out of the total 27 samples produced measurable levels of steroid, which in some cases were as high or higher than those of the 12-hr sample. Ten of these incidents occurred within the same four subjects. It seems reasonable to conclude that these levels may be due to the recovery of normal adrenal function rather than to the presence of prednisolone in the sample.

There were no significant differences among treatment average areas, either 0–12 hr or 0–24 hr (Table IV). This finding suggested that the efficiencies of absorption of prednisone, as indicated by the presence of prednisolone, did not differ significantly among treatments. The logarithm of area from 0 to 12 hr and the ratio of area divided by the dose in milligrams per kilogram times the half-life were investigated as additional measures of efficiencies of absorption, but the differences among these average parameters were not significant. The rationale for analyzing the logarithm of area was proposed by Westlake (10).

The differences among mean half-lives of prednisolone were not significant. The half-life ranged from 1.51 to 3.65 hr. The overall average half-life was 2.47 hr. None of the 24-hr points was used to calculate the half-life; the 4–12-hr points generally gave a straight line on semilogarithmic paper. The half-lives estimated are similar to those reported when prednisolone itself was administered orally (7).

CONCLUSIONS

The data presented indicate that prednisone Tablets A, B, and C differ only in the rate of appearance of prednisolone in the plasma and not in the amount of prednisone that reaches the circulation. The results document that differences in *in vivo* rates of appearance of prednisolone in humans correlate with *in vitro* rates of dissolution of prednisone and with a reported clinical failure in the use of prednisone.

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[x] To whom inquiries should be directed.