Bioavailability and Pharmacokinetics of Prednisone in Humans

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Abstract □ In a clinical study involving 22 normal adult volunteers, the bioavailability and pharmacokinetics of prednisone tablets with varying dissolution rates were evaluated. Serum levels were measured by a radioimmunoassay for prednisolone. Absorption rate constants and serum half-lives are presented. Substantial serum levels of prednisolone were attained quite rapidly (within 0.5 hr). The observed serum levels were statistically analyzed and fitted to the one-compartment open model with first-order absorption and elimination. A qualitative correlation between the *in vitro* dissolution rate and the calculated initial absorption rate constants was found. However, the *in vitro* dissolution rates were not predictive of the overall bioavailability of the prednisone tablets tested in terms of peak concentration and area under the serum concentration–time curve.

Keyphrases □ Prednisone—bioavailability and pharmacokinetics in humans, absorption rate constants and serum half-lives, 50-mg tablet compared to 10 5-mg tablets, *in vitro–in vivo* correlations discussed □ Bioavailability—prednisone in humans, 50-mg tablet compared to 10 5-mg tablets, *in vitro–in vivo* correlations discussed □ Pharmacokinetics—prednisone in humans, absorption rate constants and serum half-lives, *in vitro–in vivo* implications

With the advances in analytical technology, it is now possible to measure circulating levels of exogenous steroids (1–5). Applying the principles of pharmacokinetics to these serum levels enables one to define and quantitate the processes of absorption, distribution, metabolism, and excretion. Through the understanding of these processes, the clinician can more rationally assign optimal dosage regimens. However, this use assumes that the available drug products are reliable in terms of delivering the prescribed dose of the drug to the general circulation at essentially the same rate and extent; *i.e.*, release of the drug from the drug delivery system (capsule, tablet, *etc.*) is not variable from manufacturer to manufacturer nor from lot to lot.

Among the more important aspects associated with the rate and extent of absorption of an orally administered solid dosage form are: *(a)* the dissolution characteristics of the drug, and *(b)* the ability of the drug to permeate the GI membrane. The absorption of any given therapeutic agent could potentially be rate limited by either process. However, when several different formulations of the same drug are compared, the intrinsic permeability of the drug is usually constant for the formulations tested and the rate of solution of the solid drug may then become the major variable among the formulations.

In recent years, much emphasis has been placed on the *in vitro* screening of a drug's dissolution properties as an index of *in vivo* absorption characteristics for a given formulation. In several instances, such *in vivo–in vitro* correlations have been found (6, 7).

The purposes of this study were to: *(a)* define and quantitate the processes of absorption and elimination of prednisone after oral administration of a single 50-mg dose, and *(b)* establish the bioequivalence of a slowly dissolving 50-mg prednisone tablet to an equivalent dose of a rapidly dissolving 5-mg prednisone tablet.

Table I—Physicochemical Characteristics of Prednisone Products Evaluated

	Treatment A, Prednisone, 50-mg Tablet	Treatment B, Prednisone, 10 5-mg Tablets
Assay	48.6 mg/tablet	5.0 mg/tablet
Content uniformity:		
High	113.2% of 50 mg	99.2% of 5 mg
Low	108.6% of 50 mg	97.6% of 5 mg
Average	110.7% of 50 mg	98.2% of 5 mg
Dissolution rates:		
USP test, $T_{60\%}$, not more than 20 min at 100 rpm:		
Average, min	60.4	2.3[a]
Range, min	50.0–80.0	1.7–2.8
Percent in 20 min		
Average	34.8	100
Range	26.5–42.0	—
Disintegration time, min:		
High	1.5	2.0
Low	1.3	0.8
Average	1.5	1.0

[a] For 10 5-mg tablets.

EXPERIMENTAL

Subjects—Twenty-two healthy volunteers (15 females and seven males) averaging 25 years of age, range 19–44, with normal screening vital signs and laboratory parameters were selected. No volunteers with active peptic ulcers, tuberculosis, or psychosis were accepted. The subjects did not receive any steroid medication for 30 days and any other medication for 7 days preceding the start of the study, and they received only the medication prescribed in the protocol. The average weight of the subjects was 64.5 kg (142 lb), range 47.7–88.5 kg (range 105–195 lb).

Medication and Dosage—At zero time of each treatment period, each subject received medication as follows: Treatment A, one 50-mg tablet of prednisone[1] given as a single oral dose; and Treatment B, 10 5-mg tablets of prednisone[2] given as a single oral dose of 50 mg. Individual subjects were assigned treatments according to a randomized two-way crossover design. Seven days separated the medication day of Period I from the medication day of Period II.

Study Conditions—Subjects were required to fast overnight and for 2 hr immediately after the medication was administered. The medication was taken at zero time with 180 ml (6 fl oz) of water. No beverages (except water) were permitted during the fasting period. The subjects were not permitted to engage in any strenuous or athletic activities during the days of administration.

Sampling and Collection of Blood for Steroid Assay—Blood was taken from each subject at zero time, *i.e.*, just before dosing with each medication, and then again at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24 hr after zero time. Ten milliliters of blood was

[1] Deltasone, Upjohn Lot 16,970.
[2] Deltasone, Upjohn Lot 131AJ.

Table II—Average Serum Prednisolone Concentrations and Related Parameters Obtained from 22 Normal Adults Each Receiving Single Oral Doses of 50 mg of Prednisone

	Treatment A, Prednisone, 50-mg Tablet	Treatment B, Prednisone, 10 5-mg Tablets	Significance Level of Differences between Treatment Averages A and B
Average serum concentration, μg/100 ml, at:			
0.5 hr	40.76	57.29	$0.01 < p < 0.025$
1.0 hr	70.00	77.07	N.S.
2.0 hr	79.50	82.34	N.S.
3.0 hr	80.70	69.42	$0.01 < p < 0.025$
4.0 hr	68.63	60.60	N.S.
6.0 hr	49.35	48.01	N.S.
8.0 hr	36.04	33.07	N.S.
12.0 hr	15.30	17.41	N.S.
24.0 hr	2.06	5.21	N.S.
Peak of average serum concentration curve, μg/100 ml	80.7	82.3	N.S.
Average of individual peak serum concentration, μg/100 ml	92.6	89.4	N.S.
Average area under serum concentration curve, 0 → 12 hr, μg/100 ml × hours	573.5	559.4	N.S.
Average area under serum concentration curve, 0 → 24 hr, μg/100 ml × hours	677.6	695.1	N.S.
Average area under serum concentration curve, 0 → ∞ hr, μg/100 × hours	686.2	732.7	N.S.
Average peak time, hr	2.07	1.73	N.S.
FD/V	126.8	112.15	N.S.
Biological half-life ($t_{1/2}$), hr:			
Arithmetic mean	2.95	3.57	N.S.
Harmonic mean	2.57	2.92	N.S.
Elimination rate constant (K), hr^{-1}	0.27	0.25	N.S.
Absorption half-life ($A_{1/2}$), hr:			
Arithmetic mean	0.99	0.66	$0.025 < p < 0.05$
Harmonic mean	0.61	0.35	$0.01 < p < 0.025$
Absorption rate constant (k), hr^{-1}	1.15	1.97	$0.01 < p < 0.025$

drawn at each sampling time. Serum was harvested from all samples as soon after drawing as possible and immediately frozen.

Physical and Chemical Characteristics of Medication—The physical and chemical characteristics of the two formulations are presented in Table I. Both formulations have similar potency, content uniformity, and disintegration times, but the dissolution rates are vastly different. These dissolution rates were determined by the rotating-basket assembly of NF XIII. The USP XVII specification for dissolution of 5-mg tablets of prednisone is that 60% of the drug should dissolve in not more than 20 min.

Assay Methodology—Recent developments in radioimmunoassay procedures have produced an assay that measures circulating levels of prednisolone in serum. Since prednisone is metabolized rapidly *in vivo* to prednisolone (8, 9), the development of this assay procedure allows for the determination of the bioavailability of prednisone. The specificity and sensitivity of the prednisolone assay used in this study were reported previously (1).

RESULTS

The average serum prednisolone concentration–time relationships obtained for 22 normal adults after administration of single oral doses of 50 mg of prednisone given as either one 50-mg tablet or 10 5-mg tablets of prednisone are shown in Fig. 1. The average serum prednisolone concentrations and the related parameters of peak height concentration and area under the serum concentration–time curve, as well as a summary of the statistical analysis, are presented in Table II.

With the exception of the 0.5- and the 3.0-hr sampling times, there were no statistically significant differences between the average prednisolone concentrations attained with each treatment. The mean peak serum concentration was 89 μg/100 ml (range 45.17–175.42) for the 5-mg tablet and 93 μg/100 ml (range 50.81–157.9) for the 50-mg tablet. The 0–24-hr areas under the serum concentration–time curve were 678 and 695 μg/100 ml × hours for the 50-mg and 10 × 5-mg tablets, respectively.

Preliminary estimates of the parameters were graphically obtained by the feathering (method of residuals) technique, using the serum data plotted on semilogarithmic graph paper. Each individual subject's data were then fitted by the method of least squares, using an iterative digital computer program (NONLIN) and a digital computer[3], to conform to Scheme I (10):

$$\frac{(F)(D)}{t=0} \xrightarrow{k} \boxed{\begin{matrix} V \\ C \end{matrix}} \xrightarrow{K}$$

$$C = \frac{(F)(D)}{V}\left(\frac{k}{k-K}\right)[e^{-Kt} - e^{-kt}]$$

Scheme I

where F = fraction of dose absorbed, D = dose, k = first-order rate constant for absorption, K = first-order rate constant for elimination, C = concentration of drug in serum, and V = volume of distribution.

In the computer fitting, the graphical estimates of the parameters were used as initial estimates, and the serum concentrations were assigned equal weights. The least-squares estimates of the individual rate constants (k and K) for absorption and elimination are listed in Table III.

Both the individual and average serum concentration–time data were fitted to the one-compartment model with first-order absorption (Scheme I). In Fig. 1, the average serum prednisolone levels are plotted *versus* time, as are those predicted by Scheme I. The average rate constants used in generating these lines are listed in Table III. The r^2 and correlation coefficients between observed and predicted values for these computer-fitted data points were 0.99, indicating an excellent fit[4].

The serum half-lives calculated for prednisolone were not significantly different for either treatment, and the overall mean serum half-life from the 44 observations was approximately 3.25 hr (2.95 hr for the 50-mg tablet and 3.57 hr for the 5-mg tablets). The mean of the individuals' rate constants of elimination was 0.27 hr^{-1} for the 50-mg tablet, and it was 0.25 hr^{-1} for the 5-mg tablets. The respective mean rate constants of absorption for the 50-mg tablet and the 10 5-mg tablets were 1.15 and 1.97 hr^{-1}; their corresponding absorption half-lives were 0.99 and 0.66 hr.

[3] IBM 360/50.

[4] r^2 = (Σ squared observations − Σ squared deviations)/Σ squared observations.

Table III—Individual Pharmacokinetic Parameters Obtained from 22 Normal Adults Each Receiving Single Oral Doses of 50 mg of Prednisone (Data Fitted to One-Compartment Open Model Using a Digital Computer Programmed with NONLIN)

	Treatment A, Prednisone, 50-mg Tablet						Treatment B, Prednisone, 10 5-mg Tablets					
Subject	k, hr^{-1}	$A_{1/2}$, hr	K, hr^{-1}	$t_{1/2}$, hr	r^2	cor	k, hr^{-1}	$A_{1/2}$, hr	K, hr^{-1}	$t_{1/2}$, hr	r^2	cor
1	0.41	1.68	0.29	2.36	0.972	0.963	3.98	0.17	0.16	4.32	0.997	0.996
2	1.46	0.48	0.28	2.46	0.997	0.996	2.38	0.30	0.21	3.30	0.995	0.993
3	1.52	0.46	0.08	3.79	0.991	0.986	0.60	1.15	0.18	3.77	0.986	0.975
4	0.81	0.86	0.55	1.27	0.995	0.995	4.98	0.14	0.23	2.97	0.999	0.999
5	1.35	0.51	0.23	3.03	0.913	0.878	1.45	0.48	0.24	2.87	0.990	0.986
6	0.68	1.02	0.20	3.55	0.998	0.995	2.86	0.24	0.14	4.81	0.996	0.991
7	0.28	2.52	0.23	3.02	0.984	0.982	0.72	0.96	0.04[a]	17.58[a]	0.971	0.928
8	1.90	0.37	0.10	7.17	0.995	0.988	1.41	0.49	0.10	6.76	0.995	0.989
9	1.91	0.36	0.12	5.90	0.997	0.994	3.35	0.21	0.11	6.45	0.978	0.958
10	1.44	0.48	0.32	2.16	0.997	0.995	2.05	0.34	0.24	2.86	0.998	0.996
11	0.46	1.50	0.35	1.96	0.985	0.978	1.35	0.51	0.07	4.05	0.985	0.969
12	1.26	0.55	0.27	2.56	0.974	0.957	1.43	0.48	0.30	2.29	0.996	0.994
13	0.91	0.77	0.25	2.76	0.974	0.956	1.12	0.62	0.27	2.61	0.996	0.993
14	0.81	0.85	0.35	2.00	0.999	0.998	0.20	3.46	0.85	0.81	0.944	0.912
15	0.51	1.35	0.23	3.07	0.990	0.983	0.84	0.83	0.14	4.80	0.986	0.975
16	0.26	2.70	0.19	3.67	0.992	0.993	3.07	0.23	0.14	4.85	0.998	0.996
17	0.53	1.30	0.35	2.00	0.997	0.994	1.48	0.47	0.24	2.92	0.993	0.986
18	1.57	0.44	0.23	3.01	0.977	0.953	1.33	0.52	0.31	2.21	0.993	0.988
19	0.42	1.64	0.31	2.25	0.990	0.984	2.43	0.29	0.12	5.67	0.994	0.989
20	0.58	1.19	0.39	1.81	0.997	0.996	0.65	1.07	0.40	1.75	0.963	0.950
21	1.20	0.58	0.26	2.62	0.997	0.996	0.47	1.49	0.41	1.70	0.992	0.986
22	5.00	0.14	0.28	2.45	0.991	0.989	5.00	0.14	0.22	3.19	0.993	0.985
Mean	1.15	0.99	0.27	2.95	0.986	0.979	1.97	0.66	0.25	3.57	0.988	0.979
SD	1.01	0.47	0.10	1.77			1.40	0.52	0.17	2.14		
Average data	0.92	0.76	0.19	3.72	0.999	0.998	1.74	0.40	1.15	4.57	0.999	0.999

[a] Data not amenable to kinetic analysis.

The absorption half-life was statistically ($p < 0.05$) lower for the 5-mg tablets than for the 50-mg tablet, indicating a qualitative correlation between the *in vitro* dissolution rates and the calculated initial absorption rate constants. However, since the peak serum concentrations, the time of peak serum concentration, and the area under the serum concentration–time curve did not differ statistically, no true correlation between the bioavailability of two formulations and the *in vitro* dissolution was obtained. Hence, from a clinical view, the differences suggested by the dissolution rates were meaningless.

The rate and degree of absorption are dependent upon both the dissolution and the permeability characteristics of a drug. The prednisone from both preparations has the same permeability characteristics, and any discrepancies in bioavailability could not be dependent upon this parameter. Since the peak heights, the areas, and the peak times all indicate equivalent bioavailability (both in extent and rate of availability), it can be concluded that the presently used dissolution rate technique for prednisone is not sufficiently predictive of the commonly utilized *in vivo* bioavailability parameters. Although the pharmacokinetic analysis indicated a slower rate of absorption *in vivo* with an increase in $T_{60\%}$ *in vitro*, the peak concentration and the time of the peak serum levels of prednisolone (2.07 and 1.73 hr) were not significantly different.

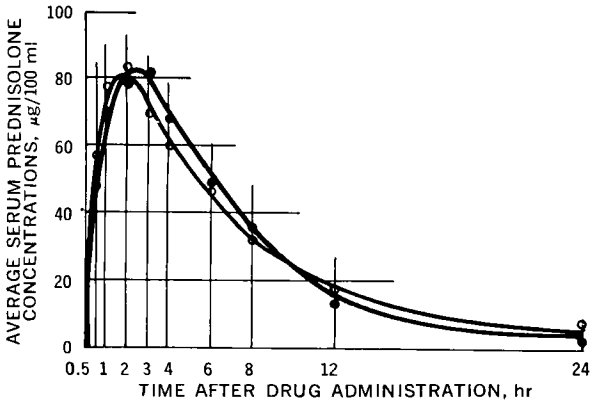

Figure 1—*Average serum prednisolone concentrations (μg/100 ml) obtained for 22 normal adults after receiving 50 mg of prednisone given as: ●, Treatment A, single oral dose of 50-mg tablet of prednisone; or ○, Treatment B, single oral dose of 10 5-mg tablets of prednisone. Lines were generated by digital computer with average parameters of Table III.*

DISCUSSION

The intent of the authors was not to take issue with the dissolution rate specifications for prednisone but rather to caution the reader in the interpretation of dissolution data. As expressed by Barr (11), *in vitro* dissolution rate measurements have been used in various ways. The two most important are: *(a)* as a sensitive quality control procedure to detect changes in the release characteristics due to lot-to-lot variation, formulation changes, or storage conditions; and *(b)* to provide an indication of differences in *in vivo* absorption characteristics of the drug and serve as a secondary standard to detect dosage forms with a potential for poor bioavailability.

Important requirements for the use of an *in vitro* dissolution method as a quality control procedure are its sensitivity, reproducibility, and ability to detect small differences in dissolution rate. These differences may or may not be indicative of differences in *in vivo* bioavailability. When *in vitro* dissolution rate methods are used to assess possible differences in the bioavailability of different formulations of the same drug, the principal requirements are a high degree of correlation between the *in vivo* and *in vitro* methods and the selectivity of the methods to reject poor products.

The fact that a tablet that did not meet compendial dissolution rate specifications was bioequivalent to a tablet with almost a 25-fold faster *in vitro* dissolution rate should be a caution against accepting the results of a battery of *in vitro* tests as criteria for *in vivo* bioequivalence of two formulations. Much of the differences observed in the dissolution rates between these tablets could possibly be attributed to the dissolution rate apparatus employed. By using a different dissolution rate apparatus or different conditions with the same apparatus, an *in vitro–in vivo* correlation that can predict prednisone bioavailability from dissolution rate data may yet be determined.

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Simple and Reliable Method for Serial Sampling of Blood from Rats

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Abstract □ A technique for serial sampling of whole blood, plasma, or serum from unanesthetized, unrestrained rats is described. This technique is sufficiently rapid, reliable, and independent of the need for elaborate preparation, specialized equipment, or practiced skills to appeal to a wide range of experimental and teaching situations requiring multiple sampling from many animals. A simple surgical procedure implants a one-piece jugular cannula cut from a commercial coil of silicone polymer tubing. Multiple blood samples are almost immediately available for 5 weeks or more. Plasma or serum samples are readily obtainable from each blood sample without transfer from the syringe in which it is collected. Intravenous injection through the cannula does not prejudice later sampling protocol.

Keyphrases □ Blood sampling, serial—simple and reliable method, technique and equipment described, rats □ Serial sampling, blood—simple and reliable method, technique and equipment described, rats

The size of the rat makes it a convenient laboratory animal for instructional classes and limited facility situations or where statistical treatment of data necessitates a number of subjects. For pharmacokinetic studies in particular, a simple technique for serial sampling of blood from these animals would be most useful.

The methods available, however, are unreliable, traumatic for the subject, difficult for the operator, or require elaborate preparation or specialized equipment. The following modification of the Popovic and Popovic (1) and Weeks and Davis (2) methods involves a simple, rapid surgical procedure, requiring neither elaborate preparation nor equipment more specialized than medical-grade tubing. Cardiac blood samples from the unanesthetized, unrestrained rat can then be taken repetitively and almost instantaneously. Plasma or serum samples are available for very little extra outlay of time or effort.

The modifications described thus eliminate problems counter to the demands of those very situations that favor choice of the rat as a laboratory subject. The technique is well suited to a wide range of experimental and teaching situations requiring multiple samples from large numbers of animals.

EXPERIMENTAL

Surgical Procedure—The rat is lightly anesthetized with ether, and the hair is removed from about a 2-cm square of the skin on the back of the neck. The animal is then held on its back by taping the forelegs to a board with adhesive tape. Light anesthesia is maintained for the rest of the surgical operation by applying a small wad of tissue paper over both the rat's nose and mouth and moistening the wad with ether occasionally.

Hair is clipped from above the right jugular vein, and a 2–3-cm skin incision is made in an anterior direction from above the midpoint of the right collar bone. By blunt dissection, about 1 cm of the external jugular vein is exposed forward from its emergence from below the collar bone. A 14-gauge hypodermic needle, from which the hub has been removed, is inserted under the skin at a point in line with, and about 1 cm anterior to, the exposed section of the jugular vein. It is passed subcutaneously but directly (*i.e.*, over the right shoulder but under the ear) to pierce the skin in the center of the clipped area on the back of the neck and just above the shoulder blades.

A 150-mm length of silicone polymer tubing is threaded through the needle, and the latter is removed through the back of the neck to leave the tubing lying subcutaneously. A syringe charged with heparinized saline is attached to the dorsal end of the tubing, and the tubing is rinsed with a little of the solution.

The exposed section of the vein is ligated anteriorly, and the ends of the tie are left uncut. By taping these ends to the operating board beside the subject's head, slight traction can be applied to the vein. A short oblique cut is made in the vein with a pair of fine scissors just below the ligature. By lifting the resultant flap with a pair of fine forceps and rotating the tubing, the ventral end of the tubing can easily be inserted into the vein. Thirty millimeters of tubing is passed toward the heart.

The tubing is secured by tying firmly around the vein just above the collar bone and again closer to the point of insertion of the can-