Comparative Bioavailability: A New Type of *In Vitro–In Vivo* Correlation Exemplified by Prednisone

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The average time to reach half-maximal plasma concentration of prednisolone and the average plasma concentrations of prednisolone at 0.5 and 1 hr obtained from three crossover bioavailability studies, involving testing of commercially available 5-mg prednisone tablets, were highly correlated (r ⪴ 0.88) with parameters derived from in vitro tablet dissolution rates performed in the spin filter apparatus of Shah. The in vitro parameters were the times to dissolve 16% or 50% of the labeled amount of prednisone or the percent of the labeled amount of prednisone dissolved in 20 min in water at 37°C. Such correlations may be useful in the setting of in vitro dissolution rate specifications for commerical prednisone tablets.

INTRODUCTION

The desirability of good correlations of variables derived from *in vitro* dissolution rates with variables derived from *in vivo* testing in human volunteers is well established in biopharmaceutics. Some good quantitative correlations have been reviewed (1).

This article presents a new type of *in vitro–in vivo* correlation based on parameters which are estimated by the logarithmic probability method of Wagner (2).

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EXPERIMENTAL

In Vitro Studies

In vitro dissolution tests were performed on ten commercial 5-mg prednisone tablets made by different manufacturers. The dissolution medium was deaerated water at 37°C, and the spin filter apparatus of Shah *et al.* (3) was used.

In Vivo Studies

Three separate bioavailability studies were performed in healthy adult male volunteers. The first study was a nine-subject three-treatment cross-over, while the second and third studies were 12-subject four-treatment crossovers. A 5-mg prednisone tablet manufactured by the Schering Corporation was used as a treatment in both the first and third studies. Doses of 10 mg of prednisone were administered orally and venous blood samples were collected. Plasma concentrations of prednisolone, the active metabolite of prednisone, were determined by a radioimmunoassay method. The details and results of these studies have been presented elsewhere (4,5); the manufacturer and lot numbers of the prednisone tablets are given in those reports.

Treatment of Data

One *in vivo* parameter was the time to reach half-maximal plasma concentration of prednisolone. The *in vitro* parameters, correlated with this *in vivo* parameter, were the times to dissolve 16% or 50% of the labeled amount of prednisone in water at 37°C using the spin filter apparatus. Both parameters were estimated by the logarithmic-probability method of Wagner (2).

The logarithmic-probability method consists of plotting percent of label dissolved, or percent of peak plasma concentration, on the probability axis of logarithmic-probability graph paper and time on the logarithmic axis. The values of the parameters can then be estimated from the equations shown below.

This procedure has been simplified by employing an electronic calculator with a program which calculates the area under the standard (normal) probability distribution function (Z) and with a second program which calculates the parameters of a linear least-square line. The percentages of label dissolved, or percentages of peak plasma concentration values, are converted to Z values, then the least-squares Z vs. log t line is calculated, where t equals time. This line has an equation of the form

$$Z = I + s \log t \tag{1}$$

where I is the intercept and s is the slope. The median (50%) point is then calculated by means of

$$t_{50\%} = \text{antilog}\,(-I/s) \qquad (2)$$

since $Z = 0$ for the median. The estimated time to dissolve 16% of label is calculated from

$$t_{16\%} = \text{antilog}\left\{\frac{-I - 1}{s}\right\} \qquad (3)$$

Correlation coefficients were calculated for the average plasma prednisolone concentration at both 0.5 and 1 hr and the percent of the labeled amount of prednisolone dissolved in water at 37°C in 20 min in the spin filter apparatus.

RESULTS AND DISCUSSION

In Vitro Studies

Table I presents the mean values of six tablets of each lot for percent of labeled amount of prednisone dissolved in the spin filter apparatus of Shah. Measurements were made at 400 rpm for 60 min, then the stirrer speed was increased to 800 rpm and absorbance readings were taken until a constant absorbance or "infinity" value was obtained. All of the tablets tested released

Table I. Mean Values of Percent of Labeled Amount of Prednisone Dissolved from 5-mg Prednisone Tablets in Spin Filter Apparatus Using Deaerated Water at 37°C

Time (min)	Mean percent dissolved from tablet									
	S	U	B	N	R	L	Mc	M	H	D
1	—	19.1	—	—	—	—	—	—	—	—
2	68.8	55.8	—	—	—	—	—	—	—	—
3	89.3	79.6	—	—	—	—	—	—	—	—
4	95.3	90.6	—	—	—	—	—	—	—	—
5	96.1	95.3	19.4	14.3	7.1	22.6	16.8	2.4	2.5	1.8
6	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
10	96.5	104	44.6	33.9	13.7	30.6	30.7	5.2	5.4	3.1
15	—	106	61.8	50.7	19.7	35.3	39.7	7.8	9.0	4.2
20	96.8	107	70.5	66.2	24.9	39.7	45.5	10.4	13.1	5.3
25	—	—	77.0	78.0	28.2	43.1	50.9	12.8	17.2	6.0
30	—	—	80.4	86.6	30.4	45.3	54.5	15.0	20.6	7.2
35	—	—	85.2	91.5	33.8	47.1	57.3	17.2	24.1	7.7
40	—	—	86.5	94.4	36.4	48.9	61.7	19.4	27.4	8.4
45	—	—	88.4	94.7	39.0	51.8	64.1	21.8	31.0	9.3
50	—	—	91.0	95.4	41.5	53.3	65.9	24.3	33.8	9.7
55	—	—	91.3	95.5	43.1	54.1	66.9	26.8	36.4	10.4
60	—	—	92.2	95.7	43.7	56.1	69.6	29.1	38.9	10.4
infinity	97.1	108	96.7	99.6	87.3	97.1	104	77.2	71.7	89.1

at least 70% of the labeled amount of prednisone at the higher stirring speed, with most tablets yielding greater than 85% of label dissolved. The dissolution rates varied greatly among the tablets tested. Tablets S and U[4] released greater than 95% of label in 5 min, while tablet D yielded only 10.4% of labeled prednisone in 1 hr. The remaining tablets had intermediate rates of dissolution.

In Vivo Studies

The results of the three bioavailability studies which were performed have been reported previously (4,5).

Correlations

An example of the logarithmic-probability graphs for the calculation of time to reach half-maximal plasma concentration is shown in Fig. 1.

[4]To our knowledge, there are four 5-mg prednisone tablets which have effective NDAs, namely those manufactured by Schering (S), Upjohn (U), Parke-Davis, and Merck, Sharp and Dohme.
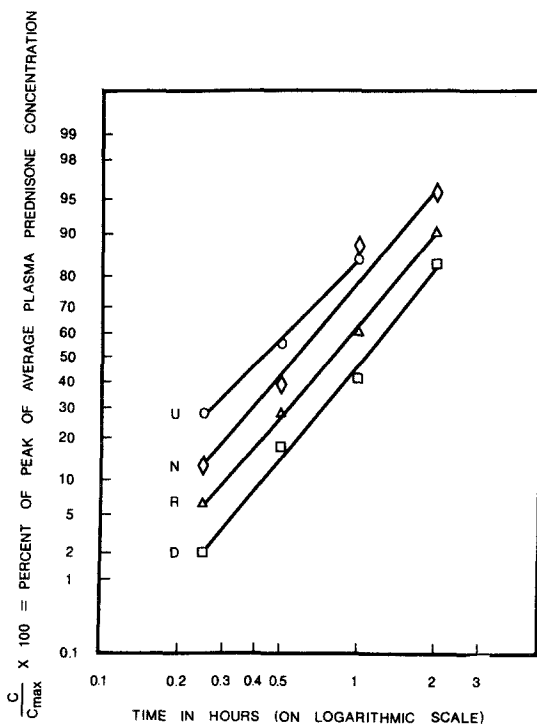
Fig. 1. Sample logarithmic-probability plot for *in vivo* absorption data.

This figure illustrates the technique with the average plasma prednisone concentrations following oral administration of four of the tablets. In the actual application of the method, the individual subject or tablet data were averaged and prednisolone, not prednisone, plasma concentrations were employed.

Table II lists the average *in vitro* and *in vivo* parameters and their 95% confidence intervals for the ten commercial 5-mg prednisone tablets tested.

The *in vitro–in vivo* correlation based on the time to reach half-maximal plasma concentrations and the time to dissolve 16% of the labeled amount of prednisone in the spin filter apparatus is shown in Fig. 2. Tablets S, U, L, B, Mc, and N gave the same *in vivo* parameter within error (i.e., the averages did not differ significantly). The average *in vivo* parameter for these six tablets is shown as the dotted line drawn through these values. This agrees with theory in that there will be some range of *in vitro* rates of dissolution

Table II. *In Vitro–In Vivo* Correlation Data for Prednisone Tablets

Study	Treatment in study	*In vitro*: spin filter (water at 37°C) time (min) to dissolve indicated % of label		*In vivo*: average time (min) to reach half-maximal plasma concentration
		16%	50%	
1	S$_1$	0.916^a	1.53	29.8^c
		(0.646–1.18)^b	(1.29–1.77)	(12.6–47.0)
3	S$_3$	same	same	24.1^d
				(11.6–31.5)
2	U	0.925	1.78	24.2^d
		(0.859–0.991)	(1.60–1.95)	(8.0–30.4)
3	B	4.13	11.7	31.4^d
		(3.74–4.52)	(8.97–14.4)	(15.2–47.7)
2	N	5.83	14.5	23.8^d
		(4.76–6.90)	(13.6–15.4)	(19.8–27.8)
2	R	12.0	80.0	39.6^d
		(9.69–14.3)	(59.0–101)	(25.2–54.0)
3	L	2.60	42.6	26.7^d
		(2.16–3.04)	(29.1–56.1)	(13.1–41.5)
3	Mc	5.14	29.7	34.1^d
		(3.09–7.19)	(13.4–46.0)	(16.4–51.7)
1	M	37.8	94.0	61.5^c
		(29.1–46.5)	(67.5–120)	(47.6–75.4)
1	H	25.7	79.9	47.2^c
		(21.5–29.9)	(58.4–101)	(34.3–55.1)
2	D	116	1918	76.8^d
		(106–126)	(1758–2078)	(54.2–99.4)

^{a}Average, based on six tablets.
^{b}95% confidence limit of the average.
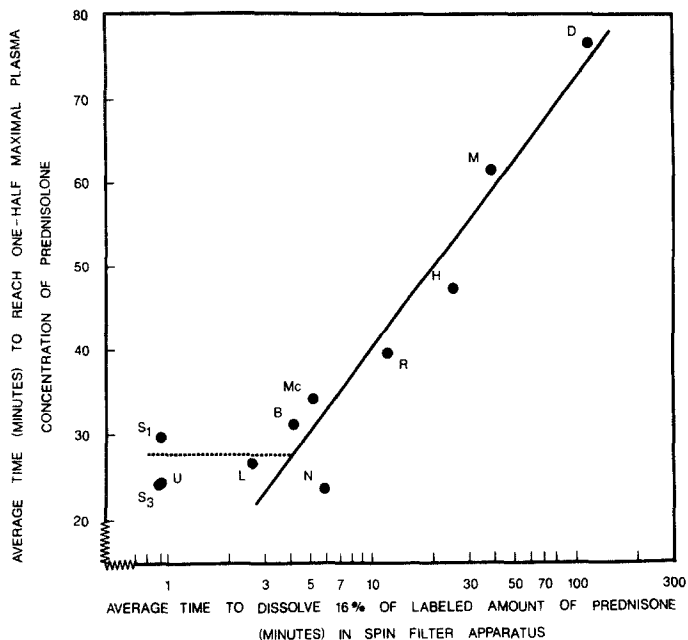^{c}Based on nine subjects.
^{d}Based on 12 subjects.

Fig. 2. Correlation of average time to reach half-maximal plasma concentration of prednisolone with average time to dissolve 16% of labeled amount prednisone in spin filter apparatus.

where the *in vivo* measurement will not differ significantly, there will be some critical value of the *in vitro* parameter, and then further decrease in rate of dissolution will cause a progressive change in the *in vivo* parameter. In this case, the critical *in vitro* rate of dissolution appears to be near that of tablet N. Further increase in the *in vitro* parameter appeared to give a linear relationship between time to reach half-maximal plasma concentration and the logarithm of the time to dissolve 16% of labeled amount of prednisone in deaerated water at 37°C in the spin filter apparatus.

The least-squares line through these points has the equation

$$\hat{Y} = 8.84 + 31.3 \log t_{16\%} \qquad (r = 0.959, p < 0.001) \qquad (4)$$

where $\hat{Y}$ is the estimated average time to reach half-maximal plasma prednisolone concentration.

The correlation of the same *in vivo* parameter with the time to dissolve 50% of the labeled amount of prednisone is shown in Fig. 3. The same phenomenon of a critical value of the *in vitro* parameter is seen, but the correlation is not as good (lower r value) as the previous one. The equation
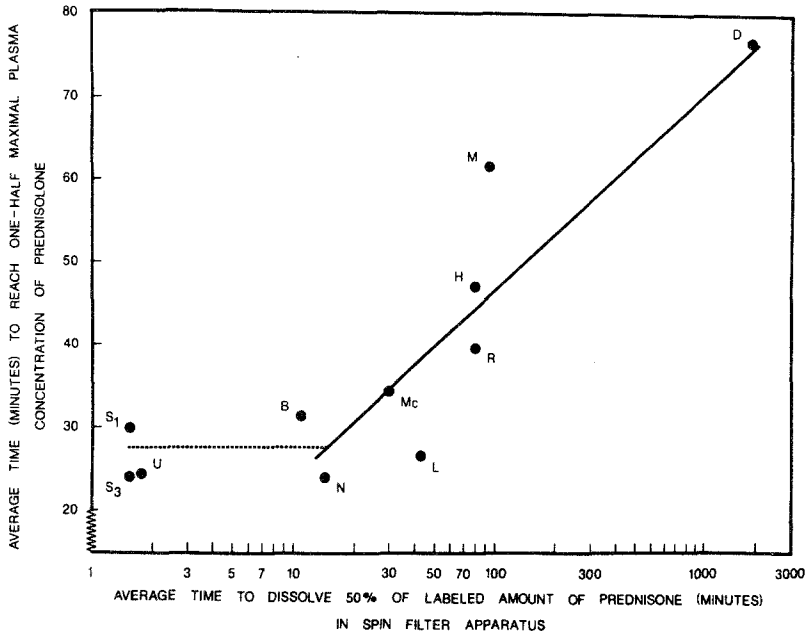
Fig. 3. Correlation of average time to reach half-maximal plasma concentration of prednisolone with average time to dissolve 50% of labeled amount of prednisone in spin filter apparatus.

(solid line) is

$$\hat{Y} = 1.18 + 22.9 \log t_{50\%} \qquad (r = 0.890, 0.001 < p < 0.01) \qquad (5)$$

Figures 4 and 5 illustrate two different types of regressions. Figure 4 is a plot of the average prednisolone plasma concentration at 0.5 hr after oral administration ($Y_{0.5}$) vs. the logarithm of the average percent of labeled amount of prednisone dissolved in 20 min ($\%D$) in deaerated water at 37°C in the spin filter apparatus. Figure 5 is a plot of the average prednisolone plasma concentration at 1 hr after oral administration (Y_1) vs. the same *in vitro* parameter as in Fig. 4. The least-squares lines drawn through the points in these two figures have the equations

$$\hat{Y}_{0.5} = 130 \log \%D - 77.5 \qquad (r = 0.915, p < 0.001) \qquad (6)$$

$$\hat{Y}_1 = 113 \log \%D + 18.9 \qquad (r = 0.879, p < 0.001) \qquad (7)$$

It is interesting that the data in Figs. 4 and 5 do not show the same criticality of the *in vitro* parameter as Figs. 2 and 3.
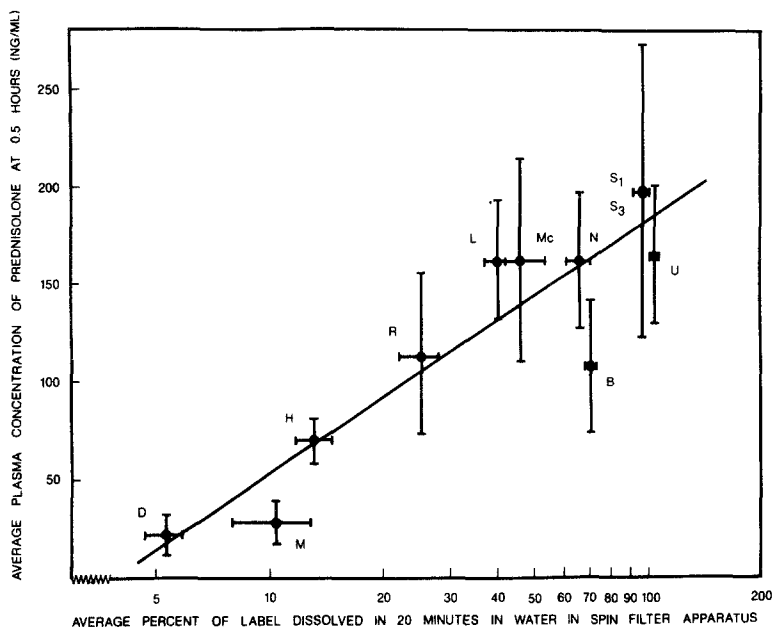
Fig. 4. Correlation of average plasma prednisolone concentration in normal man at 0.5 hr with percent of labeled amount of predinisone dissolved in 20 min in spin filter apparatus (on log scale). Bars mark off 95% confidence intervals of average.

In interpreting Figs. 1–5 it should be noted that one of tablet H or M was previously reported (4) to have failed in clinical use. Hence it would appear reasonable to us to set dissolution rate specifications for prednisone tablets using tablet N as a "cutoff" point. The upper levels of the 95% confidence intervals of the averages for these tablets are 16% dissolved in 6.9 min and 50% dissolved in 15.4 min (Table II); in 15 min the mean percent dissolved for this tablet was 50.7% and in 20 min the mean percent dissolved was 66.2% (Table I).

The National Center for Drug Analysis of the Food and Drug Administration has also obtained *in vitro* rate of dissolution data on the same prednisone tablets as those discussed here using paddle stirrer and the USP methods. Those data will be the subject of a separate report.
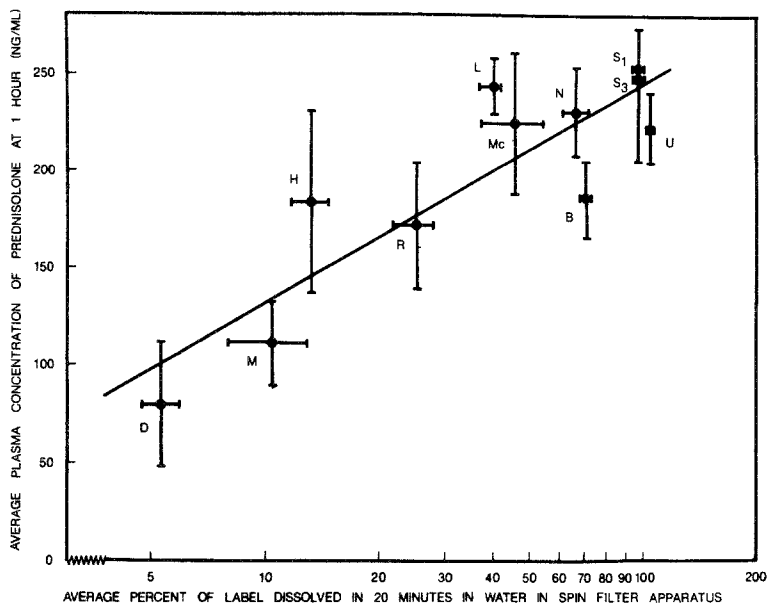
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Fig. 5. Correlation of average plasma prednisolone concentration in normal man at 1 hr with percent of labeled amount of prednisolone dissolved in 20 min in spin filter apparatus (on log scale). Bars mark off 95% confidence intervals of averages.

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