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Prednisolone Bioavailability in the Dog

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Abstract □ With a fasted dog as an animal model, the bioavailability and pharmacokinetics of prednisolone were studied following rapid intravenous injection and oral dosing of a prednisolone sodium phosphate solution and also following oral doses of prednisolone as tablets and a slurry. Hydrolysis of the phosphate ester to prednisolone in the body is extremely rapid and complete, thus permitting accurate calculation of the distribution volume of prednisolone. Enteral absorption of prednisolone from a slurry is superior to that from prednisolone tablets and from a prednisolone sodium phosphate solution. Reduced absorption from tablets, compared to the slurry, is probably due to tablet disintegration characteristics; reduced absorption from the solution is probably due to poor membrane permeability of the ionized drug. Information obtained from a single animal may indicate the need for expanded studies in humans.

Keyphrases □ Prednisolone—bioavailability and pharmacokinetics, various dosage forms compared, dogs □ Bioavailability—prednisolone, various dosage forms compared, dogs □ Pharmacokinetics—prednisolone, various dosage forms compared, dogs □ Glucocorticoids—prednisolone, bioavailability and pharmacokinetics, various dosage forms compared, dogs

Prednisolone is used primarily for its anti-inflammatory activity in several diseases. It is cleared from the body predominantly by hepatic metabolism; only about 10% of orally dosed compound is excreted unchanged in urine (1).

Peak serum prednisolone concentrations of 160 ng/ml at 0.5 hr and 298 ng/ml at 1 hr were reported following 2.5- and 5.0-mg oral doses of prednisolone, respectively, to male beagle dogs (2). Studies in humans yielded peak serum prednisolone levels at 1–2 hr following oral doses and a serum half-life of about 3 hr (3–8).

Although the influence of *in vitro* disintegration and dissolution characteristics on prednisolone pharmacokinetics was reported (5–8), no information is available on the effect of the dosage form on bioavailability characteristics.

With the beagle dog as a model system, the present study was undertaken to compare the bioavailability and pharmacokinetics of prednisolone from two different doses of a commercial tablet, a slurry, and a solution of a water-soluble prednisolone salt.

EXPERIMENTAL

A 3-year-old male beagle dog, 17.5 kg, was given 30- and 60-mg doses of prednisolone tablets[1] (oral), oral prednisolone slurry[1], and oral and intravenous prednisolone sodium phosphate solution[2] in separate experiments. All experiments were done in duplicate.

Food was withheld at least 12 hr before and throughout each experiment. During an experiment, the dog was placed in a restraining apparatus so that it could stand normally but could not disturb indwelling catheters.

In the intravenous dosing experiments, an infusion set was positioned in each front leg. Each set consisted of a 19-gauge needle with 30 cm of flexible plastic tubing with a total volume of 0.6 ml. Clotting of blood in infusion sets was prevented by infusing 2 ml of saline-diluted heparin sodium solution (10 units/ml) into the set each hour it was in position. Prednisolone sodium phosphate was administered *via* one infusion set, with injection being completed in about 30 sec. The set was flushed with 10 ml of normal saline solution and was then removed.

Blood samples (8 ml) were collected through the other infusion set shortly before dosing and at 5, 15, and 30 min and 1, 2, 4, and 6 hr after dosing. Before each sample was taken, 5 ml of residual fluid was withdrawn to remove the heparin solution in the infusion set. The blood sample was then drawn, and the volume was replaced by an 8-ml injection of normal saline. The residual fluid was reinjected followed by 2 ml of heparin solution.

Tablets were administered by placing them on the posterior portion of the tongue so that they were not fractured or chewed before being swallowed; then 50 ml of water was immediately given. The slurry was prepared by triturating prednisolone tablets to a powder and dispersing this powder in 50 ml of water. The slurry was administered directly into the dog's stomach by means of a 120-cm × 3-mm (i.d.) stomach tube. Water, 25 ml, was used to flush residual drug from the syringe and stomach tube into the stomach. The oral solution was administered directly into the stomach in identical fashion to the slurry. With all oral dosing experiments, blood samples were collected *via* an infusion set as in the intravenous case.

Blood samples were placed in heparinized tubes[3] and centrifuged at 2300 rpm for 10 min. Plasma was removed and frozen until analyzed, usually within 1 week.

Experiments were performed 3 weeks apart to avoid changes in drug pharmacokinetic parameters due to prior exposure and to ensure complete drug washout from a previous dose.

Assay of Plasma Samples—The assay used to measure prednisolone in plasma was a modification of the GLC method described by Bacon and Kokenakes (9). In the purification step by column chromatography, the volume of the first eluting solvent [benzene–acetone (9:1)] was reduced from 70 to 30 ml and the volume of the second eluting solvent [benzene–acetone (1:3)] was increased from 20 to 30 ml. This change improved separation of prednisolone from other plasma components[4].

A solution containing the silylating agent[5] was prepared by combining 0.3 ml of pyridine, 37 μl of dotriacontane (as the internal standard) in carbon tetrachloride (0.95 μg/μl), and 0.5 ml of the silylating agent. A 20-μl aliquot of this solution was added to the extracted and purified prednisolone sample in a 1-ml vial[6]. This vial was immediately sealed with a polytef-lined cap, agitated on a vortex-type mixer, and allowed to stand at room temperature for 18 hr before assay.

GLC was carried out on an instrument[7] fitted with dual flame-ionization detectors. The column was a 1.8-m × 2-mm (i.d.) U-shaped glass

[1] Meticortelone, Schering Corp., Bloomfield, N.J.

[2] Hydeltrasol, Merck Sharp and Dohme, West Point, Pa.

[3] Vacutainer, Becton-Dickinson, Rutherford, N.J.

[4] G. E. Bacon, University of Michigan Medical Center, Ann Arbor, Mich., personal communication.

[5] Tri-Sil TBT, Pierce Chemical Co., Rockford, Ill.

[6] Reacti-vial, Pierce Chemical Co., Rockford, Ill.

[7] Nuclear Chicago Selecta System model 5000, Searle, Des Plaines, Ill.

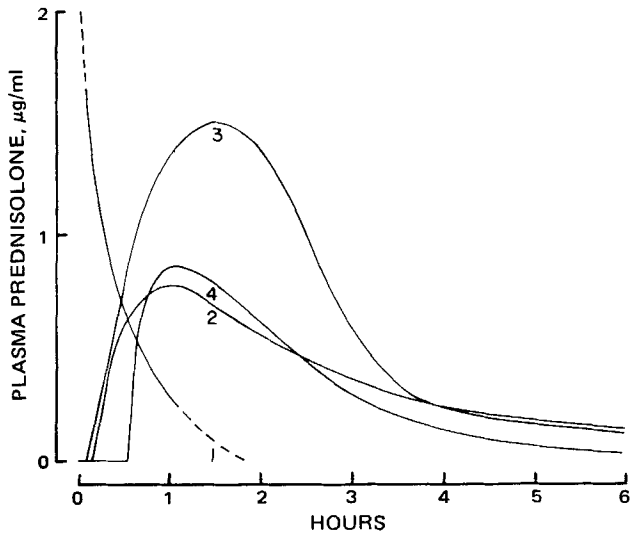

Figure 1—*Plasma prednisolone levels after doses of 30-mg prednisolone tablets (curve 4) and slurry (curve 3) and 24.7 mg of prednisolone as a prednisolone sodium phosphate oral (curve 2) and intravenous (curve 1) solution.*

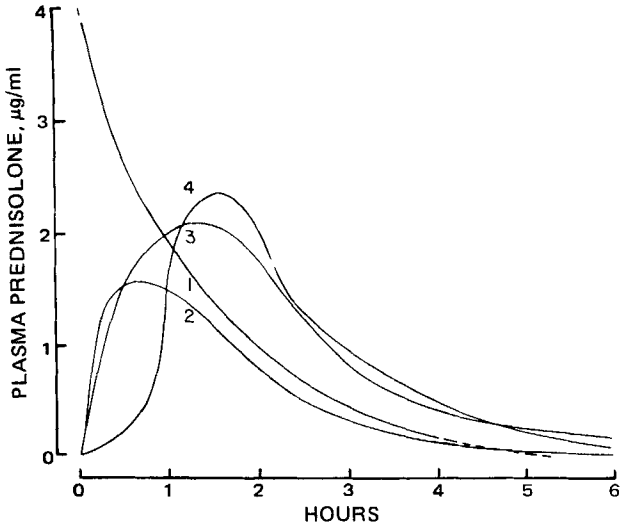

Figure 2—*Plasma prednisolone levels after doses of 60-mg prednisolone tablets (curve 4) and slurry (curve 3) and 49.3 mg of prednisolone as a prednisolone sodium phosphate oral (curve 2) and intravenous (curve 1) solution.*

tube packed with 3% OV-1 on 60–80-mesh Gas Chrom Q. The column was conditioned at 275° initially for 48 hr and then overnight after each day of sample analysis. The electrometer was set at a range of 10^{-12} amp, and the attenuator was set at 32 to give full-scale recorder deflection from an input signal of 3.2×10^{-11} amp.

Gas flow rates were 20, 25, and 200 ml/min for nitrogen, hydrogen, and air, respectively. The chromatograph was operated in the single-column mode at an isothermal column temperature of 245° with the detector bath at 300° and the injector port at 275°. Aliquots of 1 μl of solution were injected. Retention times were 341 sec for dotriacontane and 412 sec for prednisolone trimethylsilyl derivative.

Recovery of prednisolone from plasma was 68.8 ± 2.2 (*SD*) % (n = 20). A linear regression of peak area ratios of the prednisolone trimethylsilyl derivative to dotriacontane *versus* plasma prednisolone levels of 0.3–4.0 μg/ml was described by the equation $y = 1.67x - 0.01$ with $r = 1.00$.

Interpretation of Data—Semilogarithmic plots of prednisolone plasma concentrations *versus* time after intravenous doses were linear throughout the sampling period. It was, therefore, considered appropriate to analyze all data in terms of the pharmacokinetic one-compartment open model with first-order absorption and elimination. Prednisolone previously was shown to obey these types of kinetics following oral doses of prednisone tablets in humans (7).

Intravenous data were analyzed by linear regression, and oral data were analyzed on a digital computer following graphical analysis. The equation and symbolism pertaining to the one-compartment model and the method of computer analysis were described previously (10).

Pharmacokinetic parameters obtained from the three oral dosage forms were compared using the Student t test.

RESULTS

Average plasma prednisolone levels for the 30- and 60-mg doses are shown in Figs. 1 and 2. Results of pharmacokinetic and statistical analyses are presented in Tables I–III.

Individual plasma level–time curves making up the pair from each treatment were very similar, as indicated by the values of the pharmacokinetic parameters in the tables. Peak plasma prednisolone levels were obtained immediately following intravenous doses of prednisolone sodium phosphate, indicating rapid hydrolysis of the phosphate ester to prednisolone. The prednisolone elimination rate after the 60-mg injection was slower than after the 30-mg injection but was similar to that observed following oral doses (Tables I and II). The rapid elimination observed following the 30-mg doses is not clearly understood but was reproducible.

The primary objective of the intravenous doses was to obtain an accurate description of the prednisolone distribution space in the body;

Table I—Prednisolone Pharmacokinetic Parameters after a 30-mg[a] Dose to a Dog

Parameter	Intravenous Solution		Oral Solution		Slurry		Tablet	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1[b]	Run 2
k, hr^{-1}	—[c]	—	4.1 (2.6–5.5)[d]	4.2 (2.8–5.6)	1.8 (1.6–2.1)	1.7 (0.74–2.6)	3.2	4.5 (4.5–4.6)
$t_{1/2}$ (absorbance), hr	—	—	0.17	0.16	0.38	0.42	0.22	0.15
K, hr^{-1}	2.7	2.0	0.41 (0.32–0.50)	0.37 (0.30–0.45)	0.62 (0.56–0.69)	0.73[e]	0.63	0.85 (0.84–0.85)
$t_{1/2}$ (elimination), hr	0.26	0.34	1.7	1.9	1.1	0.95	1.1	0.82
FD/V^f, μg/ml	2.0	2.1	1.0 (0.92–1.2)	1.0 (0.91–1.1)	2.2 (2.0–2.4)	2.9 (2.4–3.4)	1.4	1.2 (1.21–1.23)
V, liter	12.58	12.03	—	—	—	—	—	—
t_0, hr	—	—	0.18 (0.15–0.21)	0.18 (0.15–0.21)	0.39 (0.38–0.40)	0.15 (0.05–0.26)	0.75	1.0
r^2	—	—	0.998	0.998	0.999	0.985	—	1.00
r	—	—	0.996	0.997	0.999	0.978	—	1.00
F^g	1	1	0.51	0.48	0.87	1.16	0.58	0.49
Total body clearance (VK), ml/min	560	409	82	74	124	146	126	169
Area (FD/VK), μg hr/ml	0.73	1.0	2.5(3.1)[h]	2.7(3.3)	3.5	4.0	2.3	1.5
Area (trapezoidal), μg hr/ml	—	—	2.6(3.2)	2.7(3.3)	3.4	3.8	1.6	1.5
C_{max}[i], μg/ml	—	—	0.80(0.97)	0.79(0.96)	1.3	1.5	—	0.83
t_{max}[j], hr	—	—	0.81	0.81	1.3	1.0	—	1.5

[a] For intravenous and oral solutions, 30 mg of prednisolone sodium phosphate is equivalent to 24.66 mg of prednisolone. [b] Initial graphical estimates. [c] Not relevant. [d] The 95% confidence interval. [e] Parameter fixed during computer analysis. [f] For intravenous study, $F = 1$. [g] Based on $V = 12$ liters from intravenous data. [h] Equivalent value after a 30-mg dose of prednisolone, obtained by (calculated value × 30/24.66). [i] $C_{max} = (FD/V)(k/K)^{K/(K-k)}$. [j] $t_{max} = \ln (k/K)/(k - K) + t_0$.

Table II—Prednisolone Pharmacokinetic Parameters after a 60-mg[a] Dose to a Dog

Parameter	Intravenous Solution		Oral Solution		Slurry		Tablet	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1[b]	Run 2[b]
k, hr^{-1}	—	—	5.5 (−7.9–19)	2.4 (1.2–3.6)	1.1 (0.7–1.5)	1.7 (1.2–2.1)	1.5	0.90
$t_{1/2}$ (absorbance), hr	—	—	0.13	0.29	0.62	0.42	0.47	0.77
K, hr^{-1}	0.79	0.89	0.71 (0.37–1.05)	0.72 (0.50–0.93)	0.56[c]	0.73[c]	0.66	0.82
$t_{1/2}$ (elimination), hr	0.88	0.78	0.98	0.96	1.2	1.0	1.1	0.85
FD/V, μg/ml	4.1	4.4	2.3 (1.1–3.4)	2.7 (2.0–3.3)	3.8 (3.4–4.3)	4.4 (4.0–4.8)	3.3	0.89
V, liters	12.09	11.29	—	—	—	—	—	—
t_0, hr	—	—	0.00 (−0.49–0.49)	0.03 (−0.05–0.11)	0.02 (−0.10–0.13)	0.07 (−0.10–0.16)	0.67	0.00
r^2	—	—	0.997	0.999	0.996	0.993	—	—
r	—	—	0.997	0.999	0.992	0.989	—	—
F	1	1	0.55	0.67	0.77	0.88	0.66	0.18
Total body clearance (VK), ml/min	159	167	142	144	112	146	132	164
Area (FD/VK), μg hr/ml	5.2	4.9	3.2(3.9)[d]	3.8(4.6)	6.8	6.0	5.0	1.1
Area (trapezoidal), μg hr/ml	—	—	3.1(3.8)	3.8(4.7)	6.5	5.9	3.8	4.6
C_{max}, μg/ml	—	—	1.7(2.0)	1.6(2.0)	1.9	2.3	—	—
t_{max}, hr	—	—	0.43	0.75	1.3	0.95	—	—

[a] For intravenous and oral solutions, 60 mg of prednisolone sodium phosphate is equivalent to 49.33 mg of prednisolone. [b] Initial graphical estimates. [c] Parameter fixed during computer analysis. [d] Equivalent value after a 60-mg dose of prednisolone obtained by (calculated value × 60/49.33).

volumes calculated from the zero-time intercepts of semilogarithmic plasma level–time plots were virtually identical for the four doses. The mean distribution volume was 12 liters, or 69% of total body volume, and was approximately equal to total body water. This volume was used to calculate the absolute prednisolone bioavailability in the oral experiments.

Average plasma level–time curves from oral doses showed that the slurry produced higher plasma prednisolone levels than other dosage forms following the 30-mg dose while both the slurry and tablets produced higher prednisolone levels than the solution following the 60-mg dose. The solution, however, tended to yield peak prednisolone levels somewhat earlier than the slurry and tablet doses.

In three slurry dosing experiments, computer pharmacokinetic analysis was unsatisfactory when initial graphical parameter estimates of the first-order absorption and elimination rate constants, k and K, respectively, and FD/V were allowed to float during the nonlinear fitting process. In each case, the computer generated similar values for K and k. This result can be explained in terms of the nature of the equation for the one-compartment open model:

$$C = \frac{FD}{V}\left(\frac{k}{k-K}\right)(e^{-Kt} - e^{-kt}) \qquad \text{(Eq. 1)}$$

When K and k approach a common value, $(FD/V)[k/(k-K)]$ becomes a large number while $(e^{-Kt} - e^{-kt})$ attains a diminishing value. Owing to the long word length used by the digital computer, the product of these two expressions becomes a very flexible term for fitting the plasma level–time data. However, meaningless values of k and K are generated in the process.

A similar phenomenon during two-compartment model analysis was reported previously (11, 12). To avoid this artifact, the elimination rate constant, K, was fixed at its graphically estimated value during the computer fitting. This step may be justified by the high dependability of this rate constant, compared to k and FD/V, during graphical analysis.

In three out of four plasma data sets from tablet doses, the computer prolonged the absorption lag time, t_0, and increased k to an unrealistically high value to fit the data to the model. Consequently, it was necessary to use initial graphical estimates of pharmacokinetic values instead of computer estimates in these cases. Because of the uncertainty of the kinetics associated with the absorption phase of these data, C_{max} and t_{max} were not calculated. In experiments where computer fitting was successful, r^2 and r values approached +1.0, indicating excellent agreement between the data and the proposed model.

Comparisons between Dose Levels—There were no apparent differences between k values from 30- and 60-mg doses in the solution and slurry experiments. Dose dependency of this rate constant was observed in the tablet experiments, but this result may have been due to poor description of the data by the model. Similarly, although the K values obtained from the oral solution experiments appeared higher for the 60- than for the 30-mg dose, they were almost identical for the two dose levels in the slurry and tablet experiments. This result is also true for the total body clearance, VK, since a constant distribution volume (12 liters) was used to calculate this parameter. In addition, F values for the two dose levels were virtually identical for each dosage form.

With the exception of one 60-mg tablet experiment, all areas under plasma level–time curves calculated from FD/VK were similar to those obtained using the trapezoidal rule. With the tablet and slurry doses, area values from the 60-mg dose were approximately double those from the 30-mg dose. With the solution doses, however, the 60 to 30-mg area ratios were somewhat less than 2.

The value of C_{max}, where calculated, was approximately twice as high from 60-mg doses than from 30-mg doses.

Comparisons between Dosage Forms—Since similar pharmacokinetic parameter values were obtained from the two dose levels (with the exception of K values from solution doses), parameters from the four experiments of each dosage form were combined for statistical comparison. The results (Table III) show that, of the three dosage forms studied, greatest absorption efficiency was obtained from the slurry. Areas under plasma level–time curves, normalized by dividing by the dose in prednisolone equivalents, were also larger from the slurry compared to the other treatments, but differences were significant only between the slurry and tablet treatments. No significant differences were observed in

Table III—Average Values and Results of Statistical Analysis of Pharmacokinetic Parameters for Oral Prednisolone Doses in the Dog

Parameter	Average Value[a]			t Test[b]
	Solution (1)	Slurry (2)	Tablet (3)	
F	0.55 ± 0.08[c]	0.92 ± 0.17	0.48 ± 0.21	2 > 1,3
k, hr^{-1}	4.1 ± 1.3	1.6 ± 0.3	2.5 ± 1.7	1 > 2
K, hr^{-1}	0.55 ± 0.19	0.66 ± 0.08	0.74 ± 0.11	NSD[d]
VK, ml/min	111 ± 38	132 ± 17	148 ± 22	NSD
t_{max}, hr	0.70 ± 0.18	1.13 ± 0.17	—	2 > 1
t_0, hr	0.10 ± 0.10	10.16 ± 0.16	0.61 ± 0.43	NSD
Normalized area[e] (F/VK), hr/liter	0.09 ± 0.02	0.12 ± 0.01	0.06 ± 0.03	2 > 3
Normalized area[e] (trapezoidal), hr/liter	0.09 ± 0.02	0.11 ± 0.13	0.06 ± 0.01	2 > 3

[a] $n = 4$. [b] Differences are considered significant when $p < 0.05$. [c] Standard deviation. [d] No significant differences. [e] Area divided by dose.

elimination rate constants or clearance values between treatments. The elimination half-lives were similar to those reported previously in beagle dogs (13).

DISCUSSION

Prednisolone sodium phosphate is known to produce its pharmacological action after being converted to prednisolone in the body (14). Kitagawa *et al.* (15) showed that phosphate ester hydrolysis is extremely rapid in rats, rabbits, and dogs, with peak plasma free steroid levels occurring almost immediately following intravenous injection of the ester. The monoexponential decline in plasma prednisolone levels following intravenous doses and the close similarity between the intravenous elimination rate constants and those obtained following oral doses of prednisolone indicate that the ester is rapidly and quantitatively hydrolyzed as soon as it enters the body. Any delayed hydrolysis would decrease the observed elimination rate constant. Thus, the prednisolone distribution volume of 68% total body volume, obtained following intravenous prednisolone sodium phosphate, is probably an accurate estimate of free prednisolone distribution.

Although prednisolone sodium phosphate is rapidly converted to free prednisolone *in vivo*, plasma levels following oral doses of a solution of this drug form tended to be lower than those from the slurry and tablets. Differences between solution plasma levels and those obtained from solid dosage forms were significant even when values were normalized for the slightly different doses (Table III). This result may be rationalized in terms of the ability of dissolved drug to permeate the GI membrane. Prednisolone sodium phosphate exists in solution in Forms I–III (Scheme I), depending on the solution pH.

$$\underset{\text{I}}{RH_2PO_4} \overset{K_1}{\rightleftharpoons} \underset{\text{II}}{RHPO_4^-} \overset{K_2}{\rightleftharpoons} \underset{\text{III}}{RPO_4^{2-}}$$

Scheme I

By analogy with 2-glycerolphosphoric acid, which has a pK_1 of 1.34 and a pK_2 of 6.65 (16), the pK_1 of prednisolone phosphoric acid is estimated to be ~1.5. Hence, in the stomach, with a pH range of 1–3, drug administered as III would exist mainly as either I or II. Based on a pK_1 of 1.5, the ratio of II to I species in the gastric pH range would be 0.32 at pH 1, 3.2 at pH 2, and 32.0 at pH 3. Therefore, a considerable proportion of the drug would be in the ionized form in the stomach. Since the GI epithelium is preferentially permeable to the unionized, lipid-soluble forms of most drugs (17), prednisolone sodium phosphate absorption might be expected to be hampered. Even where the pH of the stomach is as low as 1 and the drug is largely undissociated, its high water solubility and low fat solubility, compared to prednisolone, would still hinder membrane penetration. Recent studies (18) suggested that the site of prednisolone absorption is probably in the upper jejunum where the pH is close to 6. In this case, a greater percentage of drug would be in an ionized form at the absorption site, resulting in poor absorption.

The results in Tables I–III indicate that absorption of orally dosed prednisolone is somewhat reduced and delayed from tablets compared to a slurry. This result suggests, for the formulations used in this study at least, that prednisolone absorption from compressed tablets may be disintegration rate as well as dissolution rate (5) controlled. For any patient on corticosteroid therapy with demonstrated malabsorption, improved absorption from oral doses would probably be obtained by administering pulverized tablets as a slurry but not by administering a solution of a water-soluble form of the drug.

The Food and Drug Administration recommended that animal models be developed to correlate bioavailability studies in animals and humans (19). Although these results were obtained in a single animal, sufficient information has been generated to warrant expanded studies of these aspects of prednisolone bioavailability in humans.

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