Cloprednol Bioavailability in Humans

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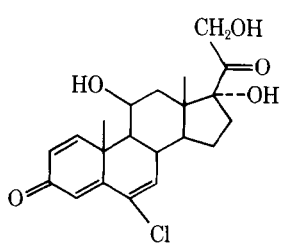
Abstract □ The bioavailability of cloprednol, a new systemic corticosteroid, was examined in a 12-subject crossover study in which two capsules, a tablet, and a solution were tested. Plasma was analyzed for cloprednol by a GLC–mass spectrometric method. The biological half-life, peak plasma concentration, peak time, plasma concentration at all sampling times, and plasma areas were evaluated for differences ($p \leq 0.05$) in comparisons of pairs among the four formulations. An analysis of variance revealed that cloprednol was absorbed to the same extent from all formulations and rapidly cleared from the plasma with a half-life of 1.86 ± 0.36 (*SD*) hr. All plasma profile parameters from the solid dose formulations were the same, demonstrating bioequivalence in both rate and extent of absorption. Significant differences were observed between the solution and solid dose formulations with respect to peak time, 15-min plasma concentration, and 0–30-min area, indicative of faster absorption from the solution; however, total plasma areas were the same for all four formulations. Comparison of plasma cloprednol levels in this study to those of a prior intravenous–oral dose study suggests that cloprednol was completely bioavailable from all formulations.

Keyphrases □ Cloprednol—bioavailability of various dosage forms in humans □ Bioavailability—cloprednol, various dosage forms in humans □ Glucocorticoids—cloprednol, bioavailability of various dosage forms in humans

Cloprednol (I), 6-chloro-11β,17α,21-trihydroxypregna-1,4,6-triene-3,20-dione, is a new systemic corticosteroid intended for oral use. Although the relative anti-inflammatory potency of cloprednol in patients with rheumatoid arthritis is approximately twice that of prednisolone, the hypothalamic–pituitary–adrenal (HPA) axis function in humans is minimally altered by cloprednol compared to equipotent anti-inflammatory doses of prednisolone, triamcinolone, dexamethasone, and betamethasone (1). Like prednisolone, cloprednol lacks the sodium-retaining properties of hydrocortisone; however, cloprednol may have less of a deleterious effect on nitrogen and calcium excretion than prednisolone (2).

The purpose of the present study was to determine the rate and extent of cloprednol absorption in humans administered various dosage forms. A tablet intended for future phase III studies was compared with two capsules used in early clinical trials and a solution in a 12-subject bioavailability study. In a separate two-subject metabolism study, cloprednol was administered intravenously and orally in solution to determine its absolute bioavailability in humans.



I

Table I—Treatment Schedule [a]

Subjects	Day 1	Day 8	Day 15	Day 22
1, 5, 9	A	B	C	D
2, 6, 10	B	C	D	A
3, 7, 11	C	D	A	B
4, 8, 12	D	A	B	C

[a] A, B, C, and D refer to the formulations described under *Experimental.*

EXPERIMENTAL

Formulations and Dosage—Formulation A was a capsule[1] containing 1.25 mg of cloprednol with starch and lactose as fillers. Formulation B was also a capsule[2] and contained 1.25 mg of cloprednol with starch, lactose, and magnesium stearate as a lubricant. Two capsules were administered to each subject.

Formulation C was a tablet[3] containing 2.50 mg of cloprednol along with starch, lactose, povidone, and magnesium stearate. One tablet was administered to each subject. Formulation D contained 2.50 mg of cloprednol in 100 ml of 15% ethanol–10% citric acid buffer.

Twelve healthy adult male volunteers received 2.5 mg of cloprednol in each of the four dosage forms in an open dose Latin-square study design (3) according to the protocol in Table I. The dose was administered at 8:00 am after a 12-hr fast and immediately followed by 200 ml of water. Subjects remained fasting for 5 hr after dosing, at which time normal meals were resumed. There was an interval of 1 week between drug administrations. Since cloprednol has a short half-life and no residual levels of drug were detected at 24 hr following a dose, 1 week was judged to be a sufficient time to assure no residual effects from prior drug administrations.

In a separate metabolism study, cloprednol was administered intravenously and orally to two subjects. Each dose contained 2.0 mg of drug dissolved in 10 ml of normal saline–ethanol (9:1). The intravenous dose was infused over 5 min, and the oral dose was diluted to 100 ml with water just prior to ingestion. Both subjects received the intravenous dose first, followed by the oral dose 2 weeks later. As already described, subjects fasted overnight, the drug was administered in the morning, and the normal diet was resumed 5 hr later.

Subjects—Subjects in the bioavailability study were normal adult male volunteers between the ages of 26 and 37 years (average 31.8 years); their body weights (69.6–87.7 kg, average 78.2 kg) were within 10% of the normal[4]. Written informed consent was obtained from each subject prior to undergoing baseline evaluation.

Study subjects did not take any medication for 2 weeks prior to the onset of and during the trial. Alcohol ingestion was not permitted from 24 hr before to 48 hr after administration of each formulation. A medical history and complete physical examination were obtained on each subject prior to the study. Routine laboratory profiles including a complete blood count, urinalysis, and chemistry panel were obtained prior to the study and 48 hr after administration of each formulation.

Subjects in the metabolism study were normal adult male volunteers, 30 and 32 years old and weighing 74.1 and 77.3 kg. Other aspects of this study were the same as already described.

Blood Sampling and Processing—In the bioavailability study, heparinized venous blood (10 ml) was obtained just prior to cloprednol administration and at 5, 15, and 30 min and 1, 1.5, 3, 4, 5, 8, 24, and 48 hr after dosing. Blood was centrifuged, and the plasma was separated and frozen prior to assay.

[1] Lot 127.
[2] Lot 144.
[3] Lot 155.
[4] As described in the table of "Desirable Weights of Adults," Metropolitan Life Insurance Co. Statistical Bulletin.

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Table II—Average Plasma Cloprednol Concentrations and Related Parameters Obtained from Normal Adult Subjects following Single Oral Doses of 2.5 mg of Cloprednol

Parameter	Treatment Average A	B	C	D	Probability (among Treatment Averages)
Plasma concentration, ng/ml, at:					
0.25 hr	3.95	1.85	5.65	16.5	<0.001
0.5 hr	33.4	33.8	35.7	43.1	0.503
1.0 hr	39.3	45.0	44.9	36.4	0.045
1.5 hr	31.2	35.7	36.1	28.4	0.017
3.0 hr	17.4	20.5	20.8	17.0	0.156
4.0 hr	12.1	12.0	13.4	10.7	0.066
5.0 hr	8.15	8.35	8.63	7.08	0.415
8.0 hr	2.75	2.41	2.13	3.24	0.377
Peak plasma concentration, ng/ml	41.3	47.0	48.8	44.2	0.378
Peak time, min	49.1	57.3	57.3	35.5	0.008
Area 0–0.5 hr, ng/ml × hr	5.16	4.68	5.87	9.50	0.005
Area 0–1 hr, ng/ml × hr	23.3	24.4	26.0	29.4	0.415
Area 0–1.5 hr, ng/ml × hr	41.0	44.5	46.3	45.5	0.700
Area 0–3 hr, ng/ml × hr	77.5	86.7	88.9	79.6	0.301
Area 0–∞ hr, ng/ml × hr	126	136	138	129	0.692
Biological half-life, hr	1.87	1.76	1.73	2.11	0.062

In the metabolism study, 15-ml blood samples were obtained following both doses. For the intravenous study, blood samples were obtained immediately upon completion of the infusion; thereafter, sampling times were the same as already described.

Assay—An appropriate amount of internal standard (6-fluoro-11β,17α,21-trihydroxypregna-4,6-diene-3,20-dione) was added to each plasma sample to be analyzed. The subsequent analysis consisted of four steps: extraction of plasma with ether (analytical reagent), partial cleanup of the extract by adsorption chromatography on an activated magnesium silicate column[5], formation of the methoxime[6]–trimethylsilyl[7] derivative, and quantitation by GLC–mass spectrometry[8].

A glass GLC column (0.6 m × 2 mm i.d.) was employed with 3% OV-1 (methyl silicone gum) on 60–80-mesh Gas Chrom Q[9]. The operational column temperature was 240°. The mass spectrometer was used in the chemical-ionization mode with methane as the carrier gas and was operated in the multiple-ion detection mode monitoring ions of *m/e* 653 (internal standard) and 667 (cloprednol). This assay was linear from 1 to 100 ng/ml of plasma with an overall coefficient of variation of ±8%.

Data Treatment—Comparisons of the four cloprednol formulations in the bioavailability study were made with respect to the following parameters: biological half-life; time to maximum plasma concentration; maximum plasma concentration; total area under the plasma concentration–time curve (*AUC*) as well as 0–30-, 60-, 90-, and 180-min areas; and plasma concentrations at 15, 30, 60, and 90 min and 3, 4, 5, and 8 hr. The biological half-life was calculated by linear regression on the 3–8-hr (log) plasma concentration values. Sequential areas under the curve were estimated by the trapezoidal rule, while the total area was estimated by the trapezoidal rule up to 8 hr and thereafter by integration using the best fitting exponential decay curve for each subject.

Although the study was of Latin-square design at inception, statistical analysis of the parameters was by randomized block design with subjects as blocks. This method was necessary since two profiles from Subject 12 were missing and one was incomplete. Therefore, none of the data obtained from Subject 12 was used, and analysis appropriate to a replicate Latin-square design was precluded. Subject 4 had one plasma profile completely missing (Formulation D), and values were substituted to include this subject in the randomized block analysis. In instances of missing samples within plasma profiles of other subjects (a total of six other plasma samples), values were substituted to permit analysis of the data. Each missing plasma concentration was estimated using:

$$x_t = (x_{t-1})(\bar{x}_t/\bar{x}_{t-1}) \qquad \text{(Eq. 1)}$$

where x_t is the missing plasma concentration at time t, x_{t-1} is the previous plasma concentration, $\bar{x}_t$ is the average (for the formulation) plasma concentration at time t for all subjects, and $\bar{x}_{t-1}$ is the average (for the formulation) previous plasma concentration for all subjects. The missing profile (Subject 4, Formulation D) was estimated as discussed in Cochran and Cox (4). After the profiles with missing plasma concentrations were "completed" and the missing profile was substituted (resulting in 44 plasma profiles for 11 subjects), statistical analysis was performed. All parameters were evaluated for significant differences ($p \leq 0.05$) in comparisons of pairs among the four formulations using Scheffe's multiple comparison test (5). Data from the two-subject metabolism studies were analyzed and compared to those of the bioavailability study without statistical analysis.

RESULTS AND DISCUSSION

In the bioavailability study, plasma samples were analyzed for cloprednol at 5, 15, and 30 min and 1, 1.5, 3, 4, 5, 8, 24, and 48 hr following each dose. At 5 min, cloprednol was detected in only two samples which contained very low levels (1.1 and 1.4 ng/ml). At 24 hr, only one sample contained measurable levels of cloprednol (3.6 ng/ml); at 48 hr, cloprednol was not detected in any sample. Therefore, the 5-min and 24- and 48-hr samples were not included in the analysis.

Average plasma cloprednol levels obtained for the formulations are plotted in Fig. 1 and are listed in Table II along with other related bioavailability parameters. At 0.25 hr, the average plasma concentration

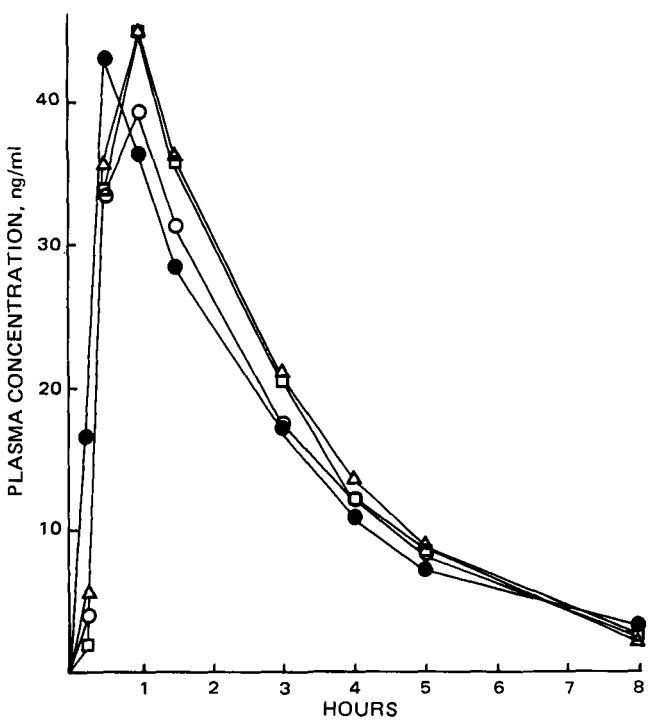

Figure 1—*Average plasma cloprednol concentrations in normal adult subjects following a 2.5-mg oral dose of cloprednol as: Formulation A (two 1.25-mg capsules) (○), Formulation B (two 1.25-mg capsules) (□), Formulation C (one 2.5-mg tablet) (△), and Formulation D (2.5 mg in solution) (●).*

[5] Florisil, J. T. Baker Chemical Co.
[6] Methoxyamine hydrochloride reagent, Eastman Kodak Co.
[7] Trimethylsilylimidazole, Pierce Chemical Co.
[8] Finnigan model 3200 with model 6000 data system.
[9] Applied Science Laboratories.

Table III—Plasma Cloprednol Concentrations Obtained from Two Normal Adult Subjects following an Intravenous and an Oral Dose of 2.0 mg of Cloprednol

Hours	Plasma Concentration, ng/ml			
	Subject 1		Subject 2	
	Intravenous	Oral	Intravenous	Oral
0.12	50.5	—[a]	50.3	—[a]
0.25	30.9	22.4	38.3	9.9
0.5	30.5	32.2	36.8	30.5
1.0	29.5	29.9	24.5	30.1
1.5	22.8	22.7	18.0	19.1
3.0	10.9	14.0	10.1	10.3
5.0	3.9	4.6	4.3	5.9
8.0	2.0	1.7	1.8	1.7
Area 0–∞ hr, ng/ml × hr	97.9	96.7	93.4	87.2

[a] No sample.

observed following a dose in solution was significantly higher (16.5 ng/ml) than that observed after any solid dosage form (1.85–5.65 ng/ml); however, there were no statistically significant plasma concentration differences among the solid dose formulations. At 0.5 hr and all subsequent sampling times, average plasma cloprednol levels were very uniform, and no statistically significant differences among any of the formulations were detected.

Average peak plasma concentrations ranged from 41.3 ng/ml following Capsule A to 48.8 ng/ml after Tablet C; however, no significant differences were detected. Plasma levels peaked earliest (35.5 min) following the solution dose, and this peak was significantly different from peak times observed after Capsule B and Tablet C (57.3 min) but not Capsule A (49.1 min). This result was expected, and the delay in onset of plasma levels following a dose in a solid formulation probably reflects the time required for disintegration and dissolution.

The *AUC* measured from 0 to 0.5 hr was greatest following the solution (9.50 ng/ml × hr) and was significantly different from that observed after Capsule A (5.16 ng/ml × hr) and Capsule B (4.68 ng/ml × hr) but not Tablet C (5.87 ng/ml × hr). All subsequent area calculations were very uniform, and no significant differences among any of the formulations were detected. The total *AUC* ranged from 126 ng/ml × hr for Capsule A to 138 ng/ml × hr following Tablet C. Since total areas were equal, all four formulations were bioequivalent with respect to amount of drug absorbed.

The average biological half-life ranged from 1.73 to 2.11 hr (mean 1.86 ± 0.36 *SD*). No significant differences in half-life were observed among any of the administered formulations.

Cloprednol, 2.0 mg, was also administered intravenously and orally in solution to two subjects. The resultant plasma concentrations and total *AUC* values are listed in Table III. Averaged plasma levels for both subjects are also plotted in Fig. 2. Initial plasma concentrations after an intravenous dose were in excess of those following oral dosing; however, by 0.5 hr, plasma levels following both administration routes were comparable. Since total plasma areas after intravenous and oral dosing were the same, it can be concluded that cloprednol was completely absorbed following an oral dose in solution (the area ratio, $area_{po}/area_{iv}$, calculated for Subject 1 was 0.99; for Subject 2, it was 0.93). The total area after intravenous dosing is compared with total areas observed in the bioavailability study in Table IV. The areas were quite similar, suggesting that cloprednol was completely absorbed from all four formulations administered in the bioavailability study.

The data also demonstrate that there was no appreciable first-pass metabolism or degradation of drug in the gut during absorption. Complete availability can be therapeutically important in patients with liver dysfunction. Variable and unpredictable blood levels of the active moiety of drugs subject to extensive hepatic metabolism during absorption, such as prednisone, have been observed in such patients (6).

These studies were carried out to demonstrate that cloprednol was bioavailable from various solid dose formulations. Furthermore, cloprednol had been administered in capsules during several early preclinical and clinical studies. Capsules A and B were designed to encompass the range of formulations used in these early studies. The tablet was designed for future use in phase III and IV studies. This study demonstrated that cloprednol capsules and tablets were equivalent in terms of the rate and extent of absorption.

No significant drug-related side effects were observed in any subject. All laboratory results for all subjects and test periods were within normal limits, except for an isolated marginally elevated uric acid in one subject noted only prior to the onset of the study and eosinophilia in another subject present before and throughout the study.

Table IV—Comparison of Total Areas following Intravenous and Oral Dosing with Cloprednol

Formulation	Total Area, ng/ml × hr
Intravenous	120[a]
A	126
B	136
C	138
D	129

[a] Total area was obtained by averaging the data from Subjects 1 and 2 in the intravenous metabolism study and multiplying by 2.5/2.0 to adjust for dose.

SUMMARY

Bioequivalence was established for Capsules A and B and Tablet C. There were no statistically significant differences among average plasma concentrations at any sampling time following administration of the two capsules or the tablet. This result illustrates the superimposition principle, which clearly shows that these formulations have equal bioavailabilities (rate and extent of absorption). Furthermore, there were no statistically significant differences in any of the other measured parameters (biological half-life, maximum plasma concentration, time to maximum concentration, and *AUC*'s) following the administration of cloprednol in these solid dose formulations.

A statistically significant difference ($p < 0.001$) between the solution and all of the solid dose formulations was observed with respect to 15-min plasma concentration, where plasma levels were higher following the dose in solution. There was a statistically significant difference ($p = 0.008$) between the solution and both Capsule B and the tablet with respect to the time of maximum concentration, with the solution dose peaking earliest. Finally, there was a significant difference ($p = 0.005$) between the solution and both capsules with respect to the 0–30-min area, with the solution giving the greatest area.

These differences reflect the fact that a drug in solution is immediately available for absorption, whereas a drug in solid form must disintegrate and dissolve prior to absorption. Bioequivalence in the amount of drug absorbed was demonstrated by equal total areas following a dose in solution, capsule, or tablet form. When total areas following intravenous and oral dosing were compared, they were shown to be equivalent, indi-

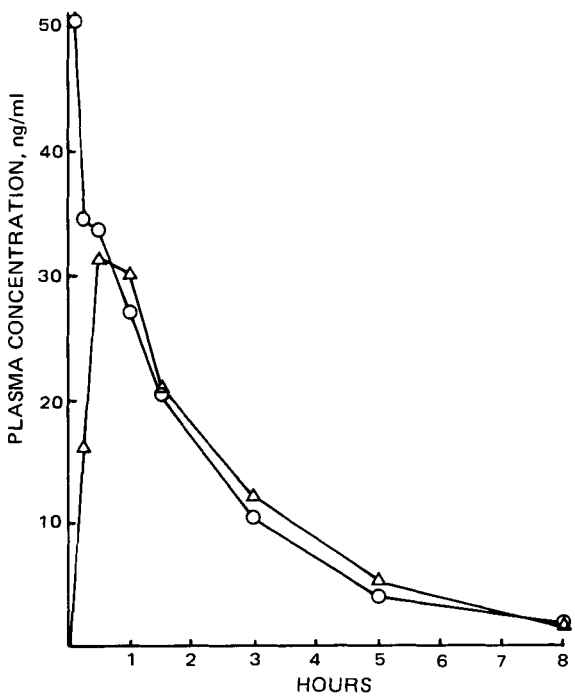

Figure 2—*Average plasma cloprednol concentrations in normal adult subjects following an intravenous (○) and oral (△) solution dose (2.0 mg) of cloprednol.*

cating that the availability of cloprednol from all of the oral dosage forms tested was complete.

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Quantitative Determination of Prednisone and Prednisolone in Human Plasma Using GLC and Chemical-Ionization Mass Spectrometry

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Abstract □ A method for the quantitative determination of prednisolone and prednisone in human plasma utilizing GLC and chemical-ionization mass spectrometry is described. Corticosteroids are extracted from plasma into ether, and the extract is purified either by passing through a magnesium silicate column or by solvent partitioning. Interference from endogenous hydrocortisone is removed by selective derivatization with Girard Reagent T. Following derivatization, prednisolone can be quantitatively separated from the water-soluble hydrocortisone derivative by simple solvent partitioning. The extracted prednisone and prednisolone are converted to their corresponding methoxyimino trimethylsilyl derivatives, and subjected to GLC–mass spectrometry. Prednisone and prednisolone plasma profiles following a 15-mg oral dose of prednisone in a human volunteer are presented. The method can measure prednisone and prednisolone in plasma at the nanogram per milliliter level.

Keyphrases □ Prednisone—GLC–mass spectrometric analysis in human plasma □ Prednisolone—GLC–mass spectrometric analysis in human plasma □ GLC–mass spectrometry—analyses, prednisone and prednisolone in human plasma □ Glucocorticoids—prednisone and prednisolone, GLC–mass spectrometric analyses in human plasma

The synthetic corticosteroid prednisone and its major metabolite prednisolone are used clinically as anti-inflammatory agents. Previously reported analytical procedures include GLC with flame-ionization detection (1), competitive protein binding assay (2), and radioimmunoassay (3, 4). The GLC method lacks sensitivity and cannot be used to follow plasma levels after the administration of a therapeutic dose.

The radioimmunoassay and competitive protein binding methods provide the required sensitivity for measurement. Specificity is apparently achieved by diluting plasma samples, thereby diluting the interference of cortisone in the prednisone assay and of hydrocortisone in the prednisolone assay. The difficulties and merits of these dilution methods recently were reviewed (5). An alternative method based on paper chromatography followed by radioimmunoassay also was described (6). Paper chromatograms require 24 hr for elution, and R_f values of the compounds of interest are obtained by comparison with R_f values of radioactive standards run on separate paper strips. Although this method has improved specificity, it is cumbersome and lengthy.

Recently, the radioimmunoassay method was used in the analysis of bioavailability and pharmacokinetic samples (7). In these studies, dexamethasone was administered to human volunteers to suppress the secretion of endogenous hydrocortisone and thus increase the specificity of the assay.

This report describes a specific and sensitive analytical method for the determination of prednisone and prednisolone in human plasma. The method is based on GLC and chemical-ionization mass spectrometry with selected ion monitoring. It can be used to study the kinetics of the interconversion of prednisone and prednisolone in humans and to acquire pharmacokinetic and bioavailability data without suppression of endogenous steroid production.

The applicability of the method is shown by the measurement of plasma prednisone and prednisolone levels in a human volunteer after oral administration of 15 mg of prednisone[1].

EXPERIMENTAL

Reagents—Methoxyamine hydrochloride[2], pyridine[2], and trimethylsilylimidazole[2] were used without further purification. Magnesium silicate[3] (60–100 mesh) and (carboxymethyl)trimethylammonium chloride hydrazide[4] (Girard Reagent T) were commercially available. Methoxyamine reagent (4% w/v) was prepared by dissolving methoxyamine hydrochloride in pyridine. Girard Reagent T (10% w/v) was prepared by dissolving it in methanol containing 1% acetic acid.

Instruments—Samples were analyzed on a gas chromatograph–mass spectrometer fitted with a chemical-ionization source and a data system[5]. Methane was used as the carrier (20 ml/min) and as the chemical-ionization reagent gas. The chemical-ionization source pressure was maintained at approximately 1 torr.

[1] Deltasone.
[2] Pierce Chemical Co., Rockford, Ill.
[3] Florisil, Matheson, Norwood, Ohio.
[4] Matheson, Norwood, Ohio.
[5] Finnigan model 3200 with model 6000 data system.

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