Suppression of Endogenous Hydrocortisone with Dexamethasone

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Abstract □ A newly developed high-pressure liquid chromatographic method was used to study the optimum dosage regimen needed to suppress endogenous hydrocortisone. Nine volunteers were randomly placed in three groups. Each group received 1 mg of dexamethasone at 11 pm (Treatment A), 2 mg of dexamethasone at 11 pm (Treatment B), or 1 mg at 11 pm and an additional 1 mg at 6 am the following day (Treatment C). Analysis of multiple blood samples obtained the day before and the day after drug administration showed suppression in all three groups. Although the duration and extent of this suppression varied, adequate suppression to permit bioavailability studies was observed for Treatments B and C.

Keyphrases □ Hydrocortisone—suppression of endogenous hydrocortisone with dexamethasone □ Dexamethasone—suppression of endogenous hydrocortisone

The principal natural glucocorticoid in humans is hydrocortisone (1). Although numerous synthetic glucocorticoids have been prepared, hydrocortisone remains an important agent in modern adrenocorticosteroid therapy, and many generic formulations have appeared on the market. However, the bioequivalence of these products has not been established. To conduct bioequivalence studies, assay methodology must be available with the required sensitivity, specificity, accuracy, and precision. A number of apparently adequate high-pressure liquid chromatographic (HPLC) methods have been reported recently (2–9).

BACKGROUND

Hydrocortisone production follows a circadian rhythm, so suppression of hydrocortisone production is necessary to simplify the interpretation of bioavailability data. Therefore, in addition to an appropriate analytical procedure, a method that will adequately suppress the endogenous hydrocortisone levels is needed. Without this suppression, it is not possible to quantitate the bioavailability of the administered hydrocortisone dosage forms. One way to achieve this suppression is to administer dexamethasone. This procedure was used in studies on prednisone (10, 11) and prednisolone (12, 13). In these cases, dexamethasone administration was necessary since endogenous steroids cross react with the antiserums used in these radioimmunoassays.

Dexamethasone is thought to act by blocking the release of endogenous ACTH (14). However, no extended detailed quantitative study has been performed to determine the amount of dexamethasone required to suppress endogenous steroids. The duration of such suppression also is not clearly established.

In one study (15), hydrocortisone levels were determined at 2 and 4 hr after administration of 1 mg of dexamethasone. During this period, hydrocortisone levels dropped to 14% of their basal values. In another study (16), the degree of suppression of endogenous hydrocortisone was related to the time of day the dexamethasone was administered. In a third study (17), 8 mg of dexamethasone was given for 2 days. The mean plasma hydrocortisone level for the subjects was 6.8 ng/ml; the blood sampling time was not given.

The present paper explores the extent and duration of dexamethasone suppression of circulating endogenous hydrocortisone using a newly developed, highly sensitive and specific HPLC assay.

EXPERIMENTAL

The nine normal male volunteers (22–45 years old) involved in this study had normal vital signs and selected laboratory parameters. The subjects, 63–80 kg, were randomly assigned to three groups of three each (Table I). Group A received 1 mg of dexamethasone, Group B received 2 mg of dexamethasone, and Group C received 2 mg of dexamethasone in two 1-mg divided doses administered 7 hr apart. Dexamethasone was given as an oral elixir[1] (0.5 mg/5 ml) in all cases.

Table I—Subject Data

Identification	Group	Height, cm	Weight, kg	Age, years
1	A	185	73	28
2	A	180	80	22
3	A	170	63	25
4	B	168	74	37
5	B	170	77	31
6	B	178	66	28
7	C	185	75	39
8	C	180	75	22
9	C	178	64	45

Basal values for plasma hydrocortisone levels were determined from heparinized blood samples (8 ml each) obtained at 8 am, 10 am, 12 noon, 2 pm, 4 pm, 6 pm, and 8 pm on Day 1 of the study. Prior to receiving the evening dexamethasone dose, the subjects were required to fast after 8 pm and to maintain that fast until noon the following day. At 11 pm on the same evening, dexamethasone (Groups A and C, 1 mg; and Group B, 2 mg) was administered to all volunteers followed by 180 ml of water. The following morning (Day 2), subjects in Group C received an additional 1 mg of dexamethasone followed by 180 ml of water at 6 am. Heparinized blood samples then were taken from all volunteers beginning at 8 am and continuing at 10 am, 12 noon, 2 pm, 4 pm, 6 pm, and 8 pm. All samples were collected, and the plasma was separated and frozen until it was assayed for hydrocortisone.

All plasma samples were assayed using a previously reported HPLC method (9) with minor modifications. An internal standard (4-pregnene-17α, 20α,21-triol-3,11-dione) was added to the plasma, which then was extracted with methylene dichloride–ether (60:40). After separation and evaporation of the organic phase, derivatization was carried out with dansylhydrazine. Upon completion of the reaction, the excess dansylhydrazine was reacted with pyruvic acid and a second extraction was performed.

The samples were placed in a water bath at room temperature and were evaporated just to dryness under a gentle nitrogen stream. The residue was immediately taken up in 200 μl of the HPLC mobile phase. Five microliters of this solution was injected[2] later onto a well-conditioned microparticulate silica column[3]. Elution[4] was carried out with an ethylene dichloride–butanol–water (93:6.6:0.4) mobile phase (1.5 ml/min). Conditioning of the column was achieved by pumping the mobile phase through the column for 1–2 days. The effluent was monitored with a fluorescence detector[5] [excitation, 240 nm; emission, 470 nm (cutoff filter)]. A linear calibration curve was found for hydrocortisone concentrations from 3 to 1000 ng/ml.

A special glassware cleaning procedure was adopted to eliminate extraneous chromatographic peaks that sometimes were observed. The procedure consisted of rinsing the glassware with distilled water (no soap was used), placing it into a commercial oven[6], which then was carried through a self-cleaning cycle, and rinsing all of the items with distilled water and methanol.

The hydrocortisone levels present in the clinical plasma samples were

[1] Decadron, lot A3240, Merck Sharp and Dohme.
[2] Model U6K sample injection valve, Waters Associates, Milford, Mass.
[3] Prepacked Hibar II column with LiChrosorb SI-60 5-μm silica, Applied Science Laboratories, State College, Pa.
[4] Model M6000A pump, Waters Associates, Milford, Mass.
[5] Model FS970, Schoeffel Instruments, Westwood, N.J.
[6] Kenmore model 423 self-cleaning oven, Sears, Roebuck and Co., Chicago, Ill.

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Table II—Basal Plasma Hydrocortisone Levels on Day 1 of Study

	Hydrocortisone Concentration, ng/ml								
	Group A			Group B			Group C		
Time	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9
8 am	116	182	150	114	162	212	208	138	78
10 am	148	218	102	126	120	182	210	164	98
Noon	56	108	108	100	94	130	92	92	86
2 pm	76	164	128	76	144	138	74	122	86
4 pm	94	120	90	62	60	100	78	88	42
6 pm	102	148	148	28	94	96	88	126	58
8 pm	44	102	24	14	46	68	42	104	28

Table III—Plasma Hydrocortisone Levels on Day 2 after Dexamethasone Administration

	Hydrocortisone Concentration, ng/ml								
	Group A			Group B			Group C		
Time	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9
8 am	7	8	12	10	10	7	16	8	16
10 am	12	7	4	7	9	7	9	6	8
Noon	24	8	18	6	9	10	7	8	11
2 pm	10	8	12	10	11	10	11	10	14
4 pm	13	7	9	11	8	8	7	7	12
6 pm	40	82	36	10	7	9	8	9	7
8 pm	14	44	14	8	36	9	8	19	9

determined from standard curves prepared by plotting the peak height ratios of the dansylhydrazones of hydrocortisone and the internal standard *versus* the concentrations of the known standards. To correct the standard curve for endogenous levels of hydrocortisone present in the control plasma, the peak height ratio of the control plasma was subtracted from all data points used in the standard curve.

RESULTS AND DISCUSSION

To establish the regimen for dexamethasone administration that would sufficiently suppress endogenous hydrocortisone production, three dexamethasone regimens were evaluated. The basal plasma levels of hydrocortisone in all nine subjects on Day 1 are recorded in Table II. There was a general trend, but not a smooth progression, of decreasing plasma levels from morning to evening. This trend agrees with the observation that plasma hydrocortisone levels follow a circadian rhythm.

The plasma levels of hydrocortisone on Day 2 are given in Table III. Substantial suppression was seen in all subjects. The plasma levels in subjects on Treatment C did not exceed 16 ng/ml from 8 am to 6 pm. Volunteers receiving Treatment B had plasma levels below 11 ng/ml throughout the same period. The plasma levels achieved with both Treatments B and C were quite stable. Suppression of endogenous hydrocortisone was not as complete in the subjects receiving Treatment A and showed greater variations. All three volunteers in Group A had plasma levels of at least 36 ng/ml by 6 pm on Day 2.

Since stress is known to affect the plasma levels of endogenous hydrocortisone (18), concern existed about the possibility of endogenous hydrocortisone levels fluctuating after suppression had been achieved with dexamethasone administration. In this study, the plasma level fluctuations seen during the control period were virtually absent in the postadministration period. This finding is important since any significant variations in the suppressed plasma hydrocortisone levels would seriously hinder the interpretation of bioavailability data of hydrocortisone dosage forms.

A profile analysis (19) using an F test for the average treatment effects, time effects, treatment with time effects, and subject effects was performed on the basal and suppressed plasma hydrocortisone levels. The data were analyzed by comparing Treatment A with Treatments B and C as well as Treatment B with Treatment C. The statistical analysis of basal plasma levels indicated that there were no significant differences due to what were evaluated as treatment effects ($p = 0.52$ and 0.90) and no significant differences due to treatment with time effects ($p = 0.29$ and 0.56). However, there were significant differences due to time ($p = 0.0001$) and intersubject variability ($p = 0.0001$), which could be expected. For the suppressed plasma hydrocortisone levels, the analysis indicated Treatment A was significantly different from Treatments B and C ($p = 0.007$). Treatments B and C were not significantly different ($p = 0.97$). A test of the treatment with time effects likewise indicated that Treatment A was significantly different from Treatments B and C ($p = 0.0001$) but that Treatments B and C were not ($p = 0.98$).

As shown in the preceding analysis, the extent and duration of the suppression of endogenous plasma hydrocortisone were similar with Treatments B and C. Therefore, selection of either regimen for subsequent bioavailability studies would be reasonable. However, since Treatment C requires an evening and a morning dose, Treatment B is more convenient. In addition, the possibility cannot be ruled out that an early morning dose of dexamethasone may affect the kinetics of orally administered hydrocortisone.

A study is underway to investigate the kinetics of hydrocortisone following a single oral dose; Treatment B administration of dexamethasone is being used.

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