PERCUTANEOUS ABSORPTION OF DEXAMETHASONE ESTIMATED BY A PLASMA RADIOIMMUNOASSAY

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Percutaneous absorption of dexamethasone and its effect on the pituitary adrenal axis were measured in vivo in normal human subjects after application to skin. Specific plasma dexamethasone and cortisol radioimmunoassays were used. Following application of 1% dexamethasone on 500 cm² of normal skin, the plasma dexamethasone concentration was maximal at 2 hr, and the average absorption was 0.25% over 8 hr; significant cortisol suppression occurred at 2, 4, and 8 hr. This technique: (1) provides an accurate assessment of the in vivo absorption of dexamethasone applied to human skin, (2) avoids exposure of the subjects to radioactive steroids, (3) permits estimation of the quantity of unmetabolized steroids absorbed, and (4) serves as a possible model for the development of similar assays for other topical steroids.

Although there are limited data on the quantity and pattern of their absorption, topical corticosteroids enjoy widespread use in clinical medicine. Previous studies of topical absorption utilized in vitro skin preparations [1], topically applied radioactively labeled corticosteroids [2–4], and physiologic changes [5–6]. These studies were limited by the inability to directly measure plasma levels of the applied steroid.

The objective of this study was to use specific radioimmunoassays to quantitate the percutaneous absorption of dexamethasone and to determine the effect of the absorbed dexamethasone on the adrenal-pituitary axis.

MATERIALS AND METHODS

Three normal males and one normal female, ages 22 to 28, participated in the study and served as their own controls. Three days before the dexamethasone study, baseline plasma cortisol concentrations were determined from samples drawn at 0, ¼, ½, 1, 2, 4, and 8 hr, beginning at 8 AM. To insure initial uniform hydration on the days that dexamethasone was applied, the applica-

Abbreviations:

IADC: integrated average dexamethasone concentration

MRC: metabolic clearance rate

T₁/₂: half time

tion sites on the subjects' backs were occluded with plastic film for 1 hr. After the occlusive wrap was removed, the excess moisture was patted dry with a paper towel and 1 ml of a standard solution of 1% dexamethasone in acetone was applied per 100 cm² (100 μg/cm²). A single application of the 1% dexamethasone solution was made at weekly intervals on areas of 100, 200, and 500 cm² in the same areas on each subject. The dexamethasone was applied with a mechanical pipette (Selectapette) and a "T" shaped glass rod was used to distribute the solution evenly. The acetone evaporated rapidly, leaving a light coat of white crystals on the skin. Following the application of the dexamethasone, the application sites were not re-occluded and there were no efforts to guard them. Blood was collected at the same times as in the control period and was assayed for dexamethasone and cortisol.

One week after the last topical study, the distribution and plasma kinetics of dexamethasone were determined by injecting 1.2 mg of dexamethasone phosphate (1 mg of dexamethasone) intravenously at 8 AM and obtaining serial plasma samples at 1/12, 1/4, 1/2, 1, 2, 4, 6, and 8 hr. The two-compartment model of Tait et al [7] was used to calculate the metabolic clearance rate (MCR), the plasma half time (T₁/₂) of elimination, and the volumes of distribution V_1 and V_2. The fraction of dexamethasone absorbed percutaneously was calculated using the following formula:

$$\text{Percent absorbed/8 hr} = \frac{\text{IADC} \times (\text{MCR}/3)}{\text{dose applied (mg)}} \times 100$$

(IADC = integrated average dexamethasone concentration).

Specific antisera were prepared for dexamethasone and cortisol by immunizing rabbits with steroid-bovine serum albumin conjugates. The methodology of the development of the assays has been described previously [8–10]. The dexamethasone assay has a sensitivity of 10 pg/assay tube and is specific for unmetabolized dexamethasone [10]. Of the known endogenous steroids, only cortisol cross-reacted significantly, 0.4%. Because of this

cross reaction, a small amount of radioactive dexametha-sone was added to each sample as an internal recovery standard, and the dexamethasone was isolated by paper chromatography before being assayed [10]. All values were corrected for the losses inherent in the extraction and chromatography procedures.

RESULTS

After application of 1 ml of 1% dexamethasone to 100 cm², the plasma dexamethasone levels were less than 20 ng/dl, and after application of 2.5 ml to 250 cm² they were from 20-40 ng/dl. Accordingly, the corresponding plasma cortisol levels were not suppressed (Fig. 1).

The mean peak plasma dexamethasone concentration, following application of 5 ml of 1% dexamethasone on 500 cm², was 250 ± 90 ng/dl and occurred at about 2 hr. This decreased over the next 6 hr to 90 ng/dl (Fig. 2). Figure 1 shows the average plasma cortisol levels during the control period and the values after application of 1% dexamethasone on 100, 250, and 500 cm². Although the initial cortisol levels of the control were higher than expected, they were reproducible. Further, the values of the 100-cm² and 250-cm² experiments reveal that all the zero time values are closely grouped. There was no significant difference between the cortisol values for the four trials at times 0, $\frac{1}{4}$, $\frac{1}{2}$, and 1 hr. Cortisol suppression was significant ($p < 0.05$, paired t-test) at 2, 4, and 8 hr after application of 1% dexamethasone on 500 cm².
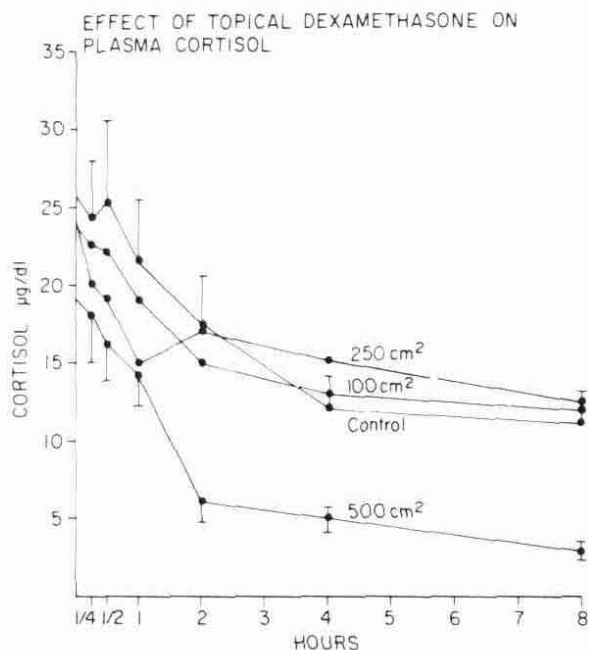
The Table summarizes the individual and aver-

FIG. 1. Mean plasma cortisol levels before and after application of 1% dexamethasone on 100, 250, and 500 cm². For clarity SEM is indicated for only the control and the 500 cm² trial. Significant suppression ($p < 0.05$) was present at 2, 4, and 8 hr after the application of 1% dexamethasone on 500 cm².
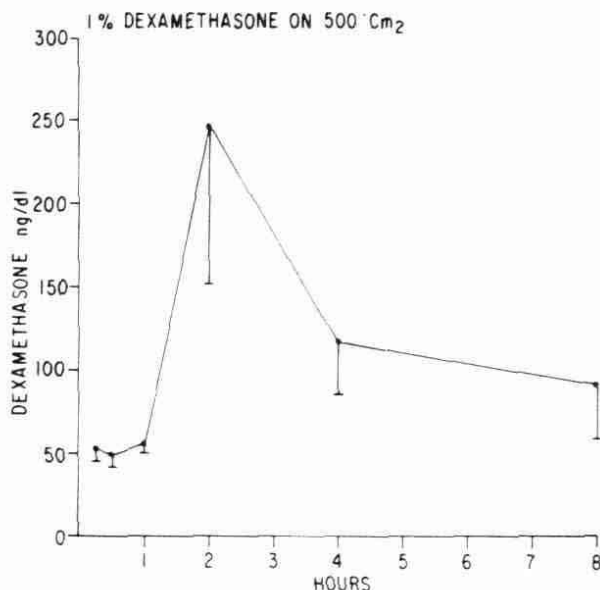

FIG. 2. Plasma concentration of dexamethasone after the application of 5 ml of a 1% solution on 500 cm², mean $\pm$ SEM.

TABLE. *Dexamethasone kinetics and percutaneous absorption*

Subjects	$T_{1/2}$ (min)[a]	MCR (liters/24 hr)[a]	% Absorption/8 hr[b]
V. Z.	280	225	0.35
G. H.	260	256	0.10
K. L.	180	504	0.34
S. W.	235	275	0.21
Mean $\pm$ SEM	238 ± 22	315 ± 64	$0.25 \pm .06$

[a] Kinetic measurements determined following the intravenous injection of 1.2 mg of dexamethasone phosphate.

[b] Percent absorbed/8 hr $= \dfrac{\text{LADC} \times (\text{MCR}/3)}{\text{dose applied (mg)}} \times 100$.

age values for the plasma $T_{1/2}$, metabolic clearance rate, and the percentage absorbed over 8 hr.

DISCUSSION

Using specific radioimmunoassays, it was possible to quantitate the percentage of unmetabolized dexamethasone absorbed, the absorption pattern of dexamethasone through normal human skin in vivo, and the effect of dexamethasone absorption on the pituitary-adrenal axis.

Although the initial hydration of the skin was constant, considerable absorption variation was noted among subjects. This is consistent with previous studies [2-3] and implies that the effective dosage range may vary greatly among patients.

Our data corroborate the study of Feldman and Maibach [2], who applied 4 μg/cm² of ¹⁴C-labeled dexamethasone to 98 cm² on the forearms of

normal volunteers and measured the excretion of radioactivity in the urine. They found that the radioactivity of the urine was maximal in the first 12 hr, 0.096% of the applied dose. Apparent differences in absorption (0.25%/8 hr in this study vs 0.096%/12 hr) may result from differences in procedures and characteristics of the respective assays. By preoccluding the application areas, the hydration of the skin was increased, possibly allowing for a more rapid and increased absorption. Theoretically, if the preocclusion had significantly affected the amount and pattern of absorption, we would expect to see a more uniform response. Some of these apparent discrepancies might also be explained by differences in experimental techniques used to quantitate absorption. With our assay we were able to determine plasma levels of the unmetabolized dexamethasone at specified frequent intervals. The measurement of radioactivity of the urine using the techniques described by Feldman and Maibach [3] did not specifically measure unmetabolized dexamethasone.

Following large doses of topical corticosteroids to normal unoccluded skin, minimal effects on cortisol secretion have been observed [6]. Using specific radioimmunoassays and applying 50 mg of dexamethasone to normal skin, we were able to correlate dexamethasone and cortisol levels and show suppression at 2, 4, and 8 hr. In previous studies, 24-hr urinary 17-OH corticosteroids were measured after the administration of topical corticosteroids; consequently, transient suppressive effects may have been undetected. We would expect greater absorption with diseased skin, along with a corresponding increase in suppression of the pituitary–adrenal axis [6–11].

No samples were collected prior to 15 min and although the lower limit of the assay is 25 ng/dl, the value at 15 min implies a small amount of absorption. Subsequent to this time more rapid absorption is seen.

The advantages of a plasma assay that is both sensitive and specific are obvious. The technique described provides an accurate assessment of the in vivo absorption of dexamethasone applied to human skin. It does not require exposure of the subjects to radioactive steroids, it permits estimation of the quantity of unmetabolized steroids absorbed, and it is a model for the development of similar assays using other topical steroids.

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