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PERCUTANEOUS ABSORPTION OF BETAMETHASONE 17-BENZOATE MEASURED BY RADIOIMMUNOASSAY

AKIRA MIZUCHI, B.S., YUKITAKA MIYACHI, M.D., KUNIHIKO TAMAKI, M.D., AND ATSUSHI KUKITA, M.D.

Institute of Biological Science (AM), Mitsui Pharmaceuticals Inc., Mobara-shi, Chiba-ken; The Third Department of Internal Medicine (YM), University of Tokyo; Department of Dermatology (KT,AK), University of Tokyo, Hongo, Tokyo, Japan

Percutaneous absorption was studied in patients following topical application of betamethasone 17-benzoate cream and gel with occlusion by means of a sensitive and specific radioimmunoassay method. Concentrations of betamethasone 17-benzoate in plasma were between 0.3 and 5 ng/ml, indicating approximately 0.05 to 0.3% of the steroid applied to the skin was detected in plasma. Plasma betamethasone 17-benzoate levels increased in proportion to the amount of the steroid applied to the skin. High correlation between plasma betamethasone 17-benzoate levels and percent inhibition of plasma cortisol was also observed. Approximately 3 ng/ml levels of betamethasone 17-benzoate in plasma induced 90% inhibition of plasma cortisol. The data suggest that betamethasone 17-benzoate in gel base was more readily absorbed than in cream base.

The value of topical corticosteroids in the treatment of various skin diseases has been well established. Information about the distribution of steroids into various body compartments has been obtained with the use of labeled compounds which permit the measurement of radioactivity in body fluids [1,2]. However, relatively little is known about plasma levels of these compounds when they are applied to the skin of patients. In this study we report plasma betamethasone 17-benzoate levels in patients following topical application of betamethasone 17-benzoate cream and gel with occlusion determined by the use of a sensitive and specific radioimmunoassay method [3]. Correlation between plasma betamethasone 17-benzoate levels and inhibition of plasma cortisol is also described.

MATERIALS AND METHODS

Complete details of materials and the betamethasone 17-benzoate radioimmunoassay procedures have been described recently [3]. They may be briefly summarized: [1,2-^{3}H]Betamethasone 17-benzoate (0.8 Ci/mmole) was synthesized in our laboratory [4]. Its purity was more than 99% as determined by thin-layer chromatography. Betamethasone 17-benzoate-21-hemisuccinate was prepared and conjugated to bovine serum albumin according to the method of Erlanger et al [5]. Male white rabbits were immunized by multiple intradermal injections of the steroid albumin conjugate suspended in complete Freund's adjuvant. Booster injections were given at 4- to 5-week intervals. Antiserum obtained 4 months after immunization was used at a final dilution of 1:14,000. Patient plasma (0.5 ml) was extracted twice with 4 ml of ethyl ether. The ether phase was then taken to dryness under N_2 and redissolved in 0.4 ml of ethyl alcohol. The radioactive recovery after ether extraction was 94 ± 4% (n = 11, mean ± SD) and no correction was made for procedural losses. Plasma extracts and standards were taken to dryness under N_2; 0.2 ml containing 2,000 dpm of [^{3}H]betamethasone 17-benzoate (0.2 ng of the steroid) was added to each sample followed by 0.5 ml of antiserum diluted to 1:10,000. The assay tubes were incubated for 18 hr at 4°C. Dextran-coated charcoal was used to separate bound from free steroid and the bound was counted in a liquid scintillation system.

Intra-assay and interassay coefficient of variations were 6.4 and 13.6%, respectively. Calculation of results was done by use of logit transformation [6]. Distilled water and plasma from subjects not receiving betamethasone 17-benzoate consistently gave blanks which were less than 0.02 ng on the standard curve (percentage binding was more than 95%) and they were defined as nondetectable. Plasma cortisol concentration was determined by radioimmunoassay [7].

Sixteen hospitalized patients were studied. Twelve were males and ages ranged from 17 to 83 years. The patients had atopic dermatitis, nummular eczema, psoriasis, and prurigo nodularis. Five, 10, or 20 gm of 0.025% betamethasone 17-benzoate in cream or gel base (supplied by Warner-Lambert Research Institute, N. J., and Mitsui Pharmaceuticals Inc., Japan) were applied evenly to the diseased skin, massaged into the skin as thoroughly as possible, and covered with plastic film. The dressing was left in place for 14 hr (6:00 PM to 8:00 AM). When the dressing was removed, the patients took a bath and the therapeutic response was recorded. The designated amount of cream or gel base without steroid was applied to the skin surface with occlusion for 4 days. Betamethasone 17-benzoate was then applied with occlusion to the same sites for 7 days and the base only without occlusion for the following 3 days. Blood was collected on days 1, 4, 7, 11, and 14 at 8:00 AM while patients were fasting.

RESULTS

Specificity of the antiserum is shown in Figure 1. This antiserum was highly specific for betametha-

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Reprint requests to: A. Mizuchi, Institute of Biological Science, Mitsui Pharmaceuticals Inc., 1900-1 Togo, Mobara-shi, Chiba-ken, Japan.

sone 17-benzoate and showed only 0.005% cross-reactivity with cortisol. The useful range of the standard curve was established between 0.05 and 5 ng (Fig. 2).

Figure 3 illustrates plasma betamethasone 17-benzoate and cortisol levels in 4 patients treated with betamethasone 17-benzoate in cream or gel with occlusion. Plasma betamethasone 17-benzoate levels were detected on days 7 and 11 when the steroid was applied. On days 1, 4, and 14, plasma betamethasone 17-benzoate levels were nondetectable. Plasma cortisol levels decreased at the 7th and 11th days. They returned to normal levels by the 14th day when the base without steroid was applied.

To correlate plasma betamethasone 17-benzoate levels and pituitary–adrenal suppression, the percent inhibition of plasma cortisol is calculated

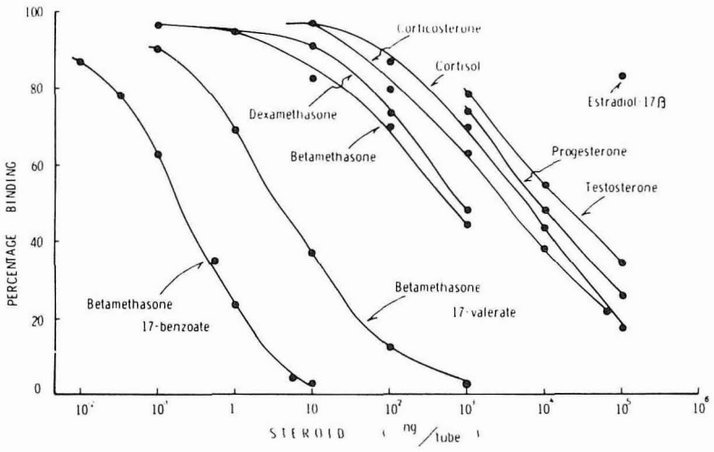

FIG. 1. Cross-reactivity of various steroids with antiserum raised against betamethasone 17-benzoate-21-hemisuccinate bovine serum albumin conjugate.

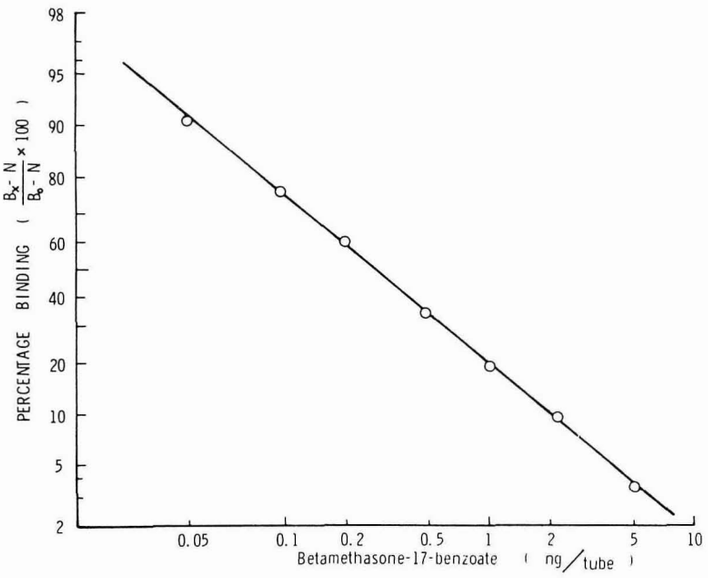

FIG. 2. Standard curve for betamethasone 17-benzoate radioimmunoassay. The abscissa is the log dose of unlabeled steroid. The ordinate is the logit transform of counts bound and is = $[(B_x - N)/(B_0 - N)] \times 100$, where B_x = counts bound at dose x, B_0 = counts bound in the absence of added unlabeled steroid, and N = the nonspecific counts bound.

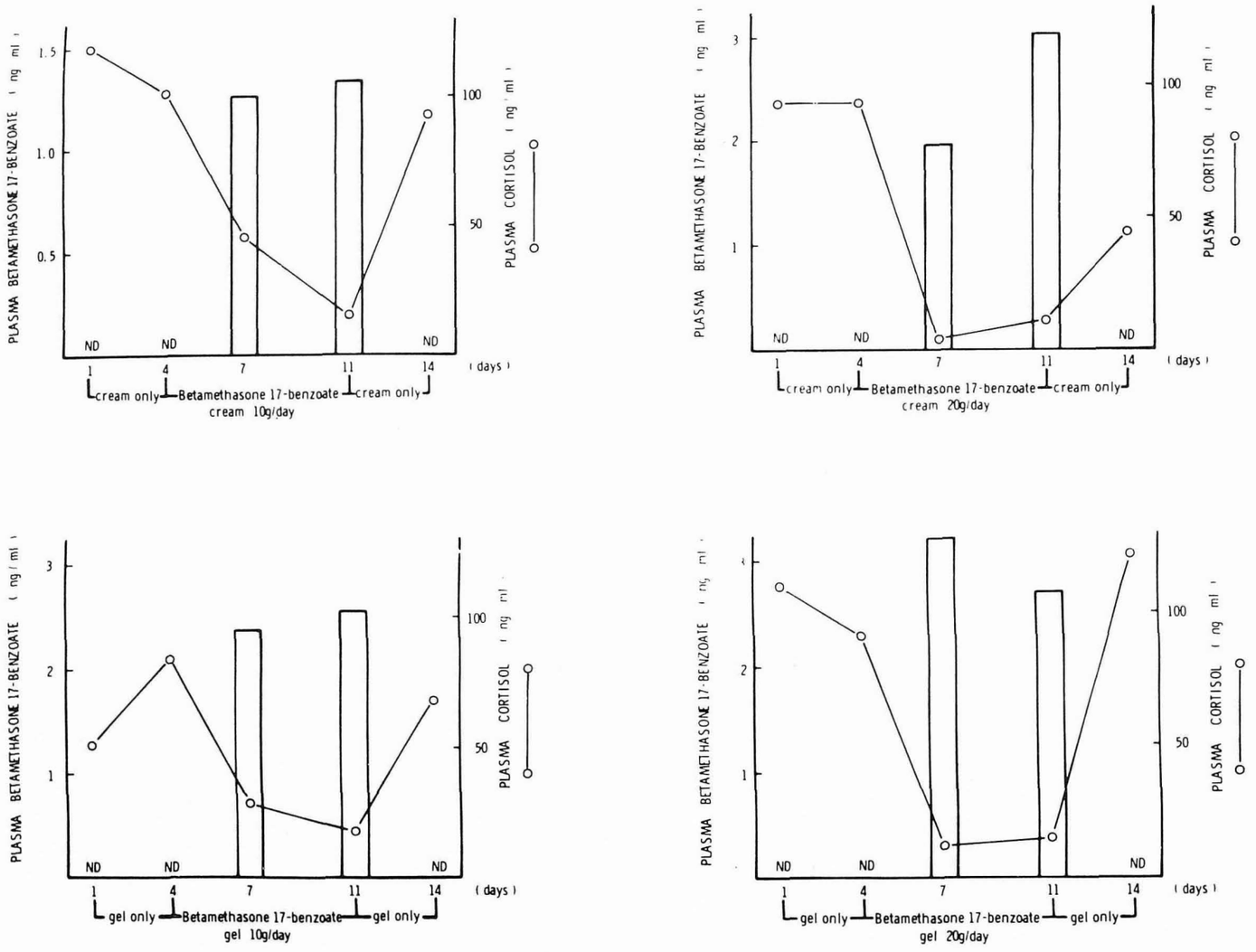

FIG. 3. Plasma betamethasone 17-benzoate and cortisol levels in 4 patients treated with 10 and 20 gm of cream and gel base with occlusion. *White column* shows plasma betamethasone 17-benzoate levels. *ND* means nondetectable. *Open circles* show plasma cortisol levels. Details are described under *Materials and Methods.*

TABLE. *Plasma betamethasone 17-benzoate levels and percent inhibition of plasma cortisol on days 7 and 11 in patients treated with betamethasone 17-benzoate cream and gel base with occlusion*

Treatment	Patient number	Day 7		Day 11	
		Plasma betamethasone 17-benzoate (*ng/ml*)	Percent inhibition of plasma cortisol[a]	Plasma betamethasone 17-benzoate (*ng/ml*)	Percent inhibition of plasma cortisol[a]
Cream	1	0.41	43.1	0.34	0.4
5 gm/day	2	0.49	19.3	0.66	11.9
Cream	3	0.91	57.6	0.59	43.8
10 gm/day	4	0.59	9.9	0.63	27.9
	5	1.26	58.4	1.38	85.8
Cream	6	1.66	57.9	2.98	20.8
20 gm/day	7	1.94	96.3	2.99	88.3
	8	2.16	35.8	2.53	91.2
Gel	9	0.66	21.6	0.64	−2.4
5 gm/day	10	1.17	42.5	1.69	43.8
	11	0.45	12.1	0.78	24.7
Gel	12	2.17	23.3	1.76	60.5
10 gm/day	13	2.37	57.1	2.54	73.4
	14	1.56	91.5	2.17	93.6
Gel	15	3.97	91.6	4.89	92.2
20 gm/day	16	3.17	87.0	2.67	85.7

[a] Obtained by $100 - \frac{(100 \times \text{plasma cortisol on day 7 or 11})}{(\text{mean levels of plasma cortisol on days 1 and 4})}$

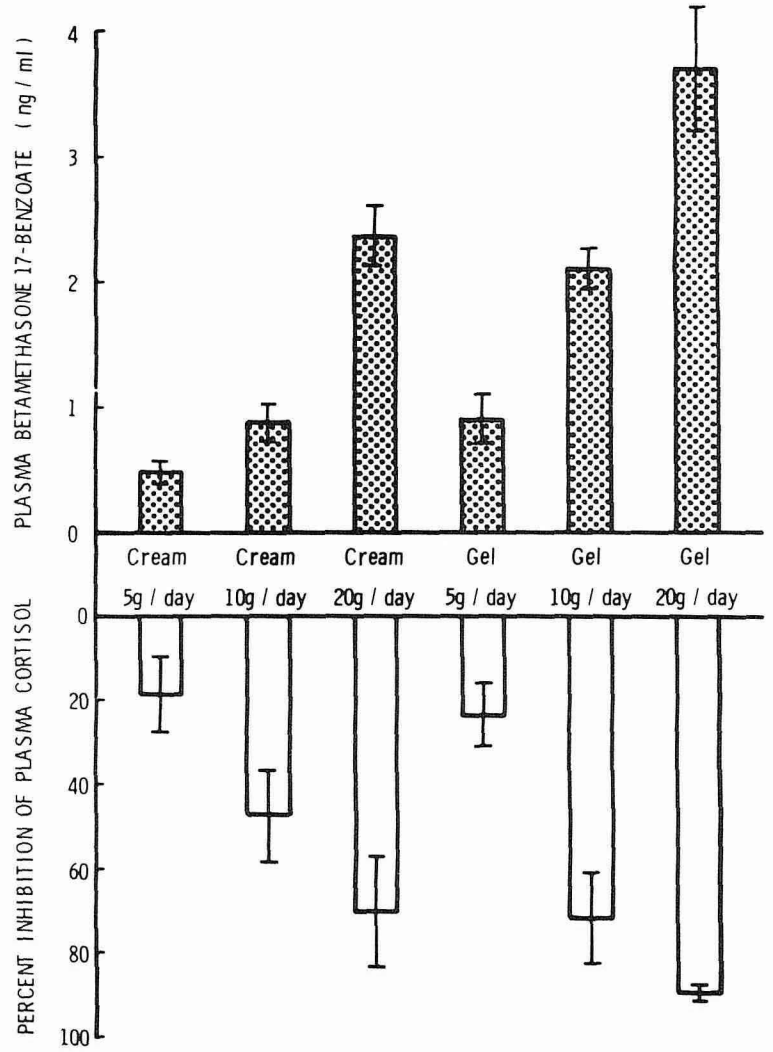

FIG. 4. Mean levels of plasma betamethasone 17-benzoate and percent inhibition of plasma cortisol at days 7 and 11 in patients treated with 5, 10, and 20 gm of cream and gel base with occlusion, respectively. The *vertical bars* indicate standard errors of the mean.

(Tab.). Shown in Figure 4 are the mean levels of plasma betamethasone 17-benzoate and the percent inhibition of plasma cortisol on days 7 and 11 in patients treated with 5, 10, and 20 gm of

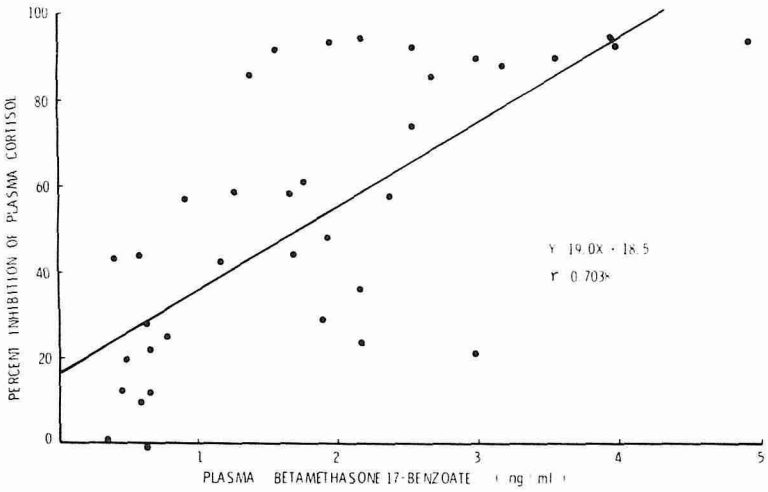

FIG. 5. Correlation between plasma betamethasone 17-benzoate levels and percent inhibition of plasma cortisol. $\gamma = 7038$, $Y = 19.0X + 18.5$, $p < 0.01$.

betamethasone 17-benzoate cream and gel base with occlusion, respectively. Plasma betamethasone 17-benzoate levels and the percent inhibition of plasma cortisol increased in proportion to the amount of the steroid applied to the skin. The data suggest that betamethasone 17-benzoate in gel base was more readily absorbed than in cream base. When 20 gm of gel base was applied to the skin, the mean level of plasma betamethasone 17-benzoate was 3.68 ng/ml. This induced approximately 90% inhibition of plasma cortisol.

When percent inhibition of plasma cortisol was plotted against plasma betamethasone 17-benzoate levels, a highly significant positive linear correlation was demonstrated ($r = 0.7038$, $Y = 19.0X + 18.5$, $p < 0.01$) (Fig. 5).

DISCUSSION

The antiserum used was highly specific for betamethasone 17-benzoate. All of the endogenous steroids tested cross-reacted less than 0.01%, indi-

cating that none of these steroids is present in sufficient concentrations in plasma to interfere with the determination of betamethasone 17-benzoate.

Studies on percutaneous absorption of a drug are complex and involve numerous factors that include the base in which the drug is incorporated, the condition of the epidermal barrier, the size of area of application, the presence or absence of occlusion, and so on. Serum steroid levels represent parameters of the flow of drug from the skin surface to excretion but not the absolute amount which has been absorbed. The data obtained suggest that there were marked differences between patients in percutaneous absorption of betamethasone 17-benzoate. Plasma betamethasone 17-benzoate levels were between 0.3 and 5 ng/ml, indicating that approximately 0.05 to 0.3% of the steroid applied to the skin was detected in plasma. A relationship between the amount of the steroid applied to the skin and plasma betamethasone 17-benzoate levels was observed.

Pituitary–adrenal suppression and decreases in endogenous cortisol levels in patients following topical application of corticosteroids have been observed [8]. Our results show that betamethasone 17-benzoate induced temporary adrenocortical suppression in proportion to the amount of the steroid applied to patients. A high correlation between plasma betamethasone 17-benzoate levels and percent inhibition of plasma cortisol was observed. Approximately 3 ng/ml of betamethasone 17-benzoate in plasma induced 90% inhibition of plasma cortisol.

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