In Vivo Percutaneous Absorption of Hydrocortisone: Multiple-Application Dosing in Man

Joseph L. Melendres,[1,2] Daniel A. W. Bucks,[1] Etienne Camel,[1] Ronald C. Wester,[1] and Howard I. Maibach[1]

Received August 6, 1991; accepted February 14, 1992

Percutaneous absorption of hydrocortisone was measured in six healthy adult men from whom informed consent had been obtained. The study compared a single topical dose to multiple-topical dose treatments (one vs three applications) on the same day. ^{14}C-Labeled hydrocortisone in acetone was applied to 2.5 cm^2 of ventral forearm skin and protected with a nonocclusive polypropylene chamber. The amount of ^{14}C measured in urine collected over 7 days was used to determine hydrocortisone absorption. The treatments, performed 2 to 3 weeks apart, each utilized adjacent sites on the same individuals. A single dose of 13.33 μg/cm^2 delivered 0.056 μg/cm^2 of hydrocortisone through the skin. When the single dose was tripled to 40 μg/cm^2, the amount delivered through the skin increased by nearly three times, from 0.056 to 0.140 μg/cm^2; the expected delivery was 3 × 0.056 μg/cm^2 = 0.168 μg/cm^2. Three serial doses of 13.33 μg/cm^2 (total, 40 μg/cm^2) were also expected to deliver 0.168 μg/cm^2 with or without soap and water washing between doses, but the observed amount of hydrocortisone delivered through the skin significantly exceeded our expectations. This indicates that multiple-dosing treatments resulted in a significant increase in bioavailability. It is postulated that increased vehicle application and washing dissolved and mobilized previously dosed hydrocortisone and increased bioavailability.

KEY WORDS: hydrocortisone; multiple dose; bioavailability; absorption; acetone; human *in vivo*.

INTRODUCTION

Topical applications of hydrocortisone and other corticosteroids frequently utilize repeated, rather than single, bolus applications of drug to the skin. It is commonly assumed that multiple applications of hydrocortisone effectively increase its bioavailability and absorption. A long-term multiple-dose rhesus monkey study by Wester *et al.* indicated that this was true (1). However, short-term experiments in the rhesus monkey by Wester *et al.* and long-term pharmacokinetic assays by Bucks *et al.* did not show an increase in hydrocortisone absorption following multiple dosing (2,3). The following investigation was designed to determine if multiple-dose therapy (dosing the same site three times in the same day) would increase drug bioavailability in human skin.

MATERIALS AND METHODS

In each procedure in this crossover study, the subjects were six healthy male volunteers, 25–85 years old, from whom informed consent had been obtained. The treatments were performed on two adjacent sites on each forearm (four sites total). Each site received a different treatment; each was performed 2 to 3 weeks apart, alternating forearms between the treatments, to allow for systemic and dermal clearance of residual radioactivity.

[4-^{14}C]Hydrocortisone purchased from Research Products International (Mount Prospect, IL) was administered in three acetone formulations produced by dissolving a predetermined amount of unlabeled crystalline hydrocortisone (Sigma Chemicals, St. Louis, MO) in acetone and mixing with an appropriate amount of ^{14}C-labeled material. Twenty microliters of test material was applied per application to 2.5 cm^2 of ventral forearm skin and protected by a modified nonocclusive, complete 25-mm polypropylene chamber (Hilltop Research, Inc., Miamiville, OH) (4). The chamber adhered to the skin by application of an adhesive dressing (Tegaderm, 3M Medical Surgical Division, St. Paul, MN) to the periphery of the complete chamber. The test material was washed off the treated areas five times using two cotton balls dipped in 50% soap–water alternated with three dipped in water. Four methods of application and removal (treatments 1, 2, 3, and 4) were each performed 2 to 3 weeks apart, alternating adjacent left and right forearm sites of each of the six subjects.

Treatment 1—One bolus application of 1.0 μCi/13.33 μg/cm^2 on the right arm, 3 in. from the antecubital fossa. The dose was exposed to the skin for 24 hr, followed by removal by washing, and the chamber was replaced with a new one.

Treatment 2—One bolus application of 1.0 μCi/40.0 μg/cm^2 on the left arm, 3 in. from the antecubital fossa. The dose was exposed to the skin for 24 hr, followed by removal by washing, and the chamber was replaced with a new one.

Treatment 3—Three repeat applications of 0.33 μCi/13.33 μg/cm^2 on the left ventral forearm 1 in. from the antecubital fossa. One dose was applied, followed by identical doses 5 and 12 hr after the initial dose. The site was washed and the chamber replaced with a new one 24 hr after the initial dose was applied.

Treatment 4—Three repeat applications of 0.33 μCi/13.33 μg/cm^2 on the right ventral forearm, 1 in. from the antecubital fossa. One dose was applied, followed by identical doses 5 and 12 hr after the initial dose, and the previous dose was washed before application of the subsequent dose. A third wash was performed and the chamber replaced with a new one 24 hr after the initial dose was applied.

Note that in all procedures, the same protective chamber was used until 24 hr after the initial administration of the drug, at which time it was replaced with a new one. In each treatment, a final wash, chamber collection, and skin tape stripping with cellophane tape (Scotchbrand, 3M, St. Paul, MN) were performed seven days after the initial application of hydrocortisone. Wash samples, cellophane tape strips, chambers, and urine were collected to determine dose accountability and mass balance. Urine was collected and measured every 24 hr for 7 days and duplicate 5-ml aliquots were collected and combined with 10 ml of liquid scintillation cocktail (Universol ES, ICN Biomedicals, Costa Mesa, CA). ^{14}C content was measured using a liquid scintillation counter

[1] University of California School of Medicine, Department of Dermatology, Box 0989, San Francisco, California 94143–0989.
[2] To whom correspondence should be addressed.

0724-8741/92/0900-1164$06.50/0

Table I. Hydrocortisone Dosing Sequence and Mass Absorbed

Treatment	Dose per application ($\mu g/cm^2$)	Cummulative dose ($\mu g/cm^2$)	Total vehicle volume (μl of acetone)	Total mass absorbed ($\mu g/cm^2$)[a]
1[b]	13.33	13.33	20	0.056 ± 0.073
2[c]	40.00	40.00	20	0.140 ± 0.136
3[d]	13.33	40.00	60	0.372 ± 0.304
4[e]	13.33	40.00	60	0.472 ± 0.396

[a] Mass absorbed per square centimeter of skin. The values are the means ± SD of six volunteers.
[b] Single dose of 13.33 $\mu g/cm^2$, administered in 20 μl of acetone.
[c] Single dose of 40.0 $\mu g/cm^2$, administered in 20 μl of acetone.
[d] Three serial 13.33 $\mu g/cm^2$ doses, each administered in 20 μl of acetone.
[e] Three serial 13.33 $\mu g/cm^2$ doses, each administered in 20 μl of acetone, washed before application of the subsequent dose.

(Packard 4640, Arlington Heights, IL). Mass, dose sequence (Table I), and percentage recovery of cumulative dose (Table II) were calculated. The percentage of the dose excreted was calculated from the amount recovered in the urine. The urinary excretion data were corrected for radiolabeled hydrocortisone clearance through other routes by inclusion of intravenous data (mean = 76.5%) from previously reported rhesus monkey experiments. This was accomplished by dividing the average percentage of the applied dose recovered in the subjects' urine by the average percentage of the applied dose recovered in urine from rhesus monkeys following intravenous administration of radiolabeled hydrocortisone (5,6). Percentage excretion was based upon the total amount of hydrocortisone applied during the first 24 hr; specifically, the percentage of the single dose applied or the percentage from the cumulative total of the multiple doses applied, wherever appropriate. All data were calculated to percentage absorption and mass of hydrocortisone absorbed based on penetration through a 2.5-cm^2 area.

The study was specifically designed to compare a single low dose (13.33 $\mu g/cm^2$) to a single larger dose (40 $\mu g/cm^2$; three times the amount) and to two multiple-application therapy (13.33 $\mu g/cm^2 \times 3 = 40$ $\mu g/cm^2$) treatments. Student's two-tailed, paired t tests were employed to compare the percentage of the applied dose absorbed and observed mass absorbed per square centimeter between each of the treatments.

RESULTS

Hydrocortisone percutaneous absorption is expressed as micrograms of hydrocortisone recovered per square centimeter in Table I and as percentage recovered from urine in Table II. Table III provides expected and observed hydrocortisone absorption values as micrograms per square centimeter from each treatment. Comparison of observed and expected hydrocortisone mass absorbed from each treatment is illustrated in Fig. 1. Table II shows that the observed mass absorbed from 13.33 $\mu g/cm^2$ in 20 μl acetone during treatment 1 is 0.056 $\mu g/cm^2$. A threefold dose increase, accompanied by the same vehicle volume (20 μl of acetone), was expected to yield 3×0.056 $\mu g/cm^2$ or 0.168 $\mu g/cm^2$ (treatment 2). This value lies within the mean ± SD of the observed mass observed (0.140 $\mu g/cm^2$ ± 0.136). During multiple-dose treatments, it was expected that hydrocortisone absorption would also be 0.168 $\mu g/cm^2$. Consequently, the observed mass of hydrocortisone absorbed for treatment 3 is approximately double (0.372 ± 0.304 $\mu g/cm^2$; $P < 0.05$) and almost triple (0.472 ± 0.396 $\mu g/cm^2$; $P < 0.05$) for treatment 4. Note that single-dose treatments 1 and 2 differ from multiple-dose treatments 3 and 4 by the amount of vehicle applied. Table I shows that during multiple-dose treatments 3 and 4 the subjects received 60 μl rather than 20 μl of acetone. Treatment 4 differs from treatment 3 in that the skin was washed at three time intervals during the first 24 hr with soap and water. Of interest, hydrocortisone absorption was not significantly different ($P > 0.05$) between these treatments, although the application site was washed before application of subsequent doses. Hence, the data indicate that hydrocortisone absorption was increased by washing and an increase in acetone volume. Statistical significance between treatments is summarized in Table IV.

DISCUSSION

The intention of this study was to determine if multiple-dose therapy could increase hydrocortisone bioavailability in human skin. The following discussion of this study's re-

Table II. Percentage Recovery of Applied Dose[a]

Treatment	Amount absorbed[b]	Surface washes	Polypropylene chambers	Cellophane tape strips	Total
1	0.35 ± 0.34	59.15 ± 22.76	16.88 ± 4.61	0.31 ± 0.18	76.69 ± 17.65
2	0.42 ± 0.55	50.48 ± 21.05	21.68 ± 6.49	0.49 ± 0.22	73.07 ± 13.07
3	0.93 ± 0.76	49.56 ± 19.72	24.00 ± 4.25	0.78 ± 0.56	75.27 ± 16.98
4	1.18 ± 0.99	65.87 ± 12.11	19.50 ± 3.27	0.36 ± 0.13	86.91 ± 7.05

[a] The values are mean percentage recovery ± SD of the total applied dose to the skin ($n = 6$).
[b] The amount absorbed was calculated from recovery of radiolabeled hydrocortisone in the urine collected over 168 hr and corrected for other routes of excretion.

Table III. Expected and Observed Hydrocortisone Absorption

Treatment	Dosing sequence	Hydrocortisone absorbed ($\mu g/cm^2$)[a] Expected	Observed
1	13.33 $\mu g/cm^2 \times 1$	—	0.056
2	40.0 $\mu g/cm^2 \times 1$	0.168[b]	0.140
3	13.33 $\mu g/cm^2 \times 3$ (no wash)	0.168[b]	0.372
4	13.33 $\mu g/cm^2 \times 3$ (wash)	0.168[b]	0.472

[a] The values are the average amount absorbed from six volunteers.
[b] Expected values are 0.056 $\mu g/cm^2 \times 3 = 0.168$ $\mu g/cm^2$.

sults details how our data support the common clinical belief that multiple dosing of human skin can increase hydrocortisone bioavailability.

The percentage absorption of hydrocortisone was not significantly increased by an increase in single-dose concentration. Specifically, tripling the baseline hydrocortisone concentration tripled the mass absorbed (Table I), but it did not significantly increase the percentage absorbed ($P > 0.05$) (Table II).

Unlike a single 40.0 $\mu g/cm^2$ dose (treatment 2), 40.0 μg of hydrocortisone is not entirely available to each centimeter of skin during the first 24 hr of either multiple-application treatment (3 or 4). In treatment 3, only 13.33 μg was available to each centimeter of skin during the first 5 hr, 26.66 μg (2×13.33 μg) was available to each centimeter of skin during the following 7 hr, and 40.0 μg (3×13.33 μg) was available to each centimeter of skin during the final 12 hr; even less hydrocortisone was available to the skin after each application during treatment 4 because the previous dose was washed off. Nevertheless, absorption of the drug had significantly increased, past our expectations. This observation may be partially explained by examining the total vehicle volume that accompanied the total applied dose as described in Table I. Clearly, 60 μl of acetone accompanied 40 μg of hydrocortisone during repeat application treatments,

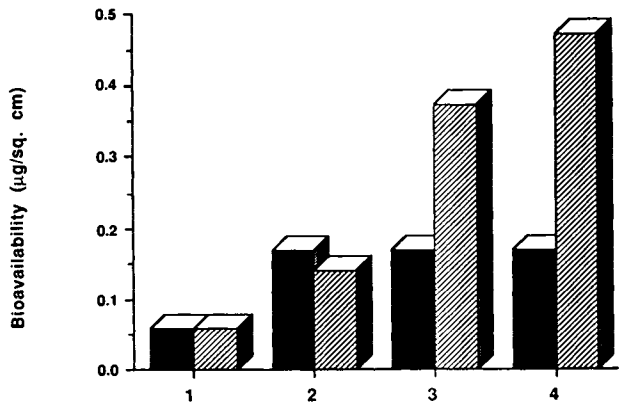

Fig. 1. Expected and observed hydrocortisone *in vivo* drug delivery to human volunteers with single dosing and triple therapy (three doses in a single day): 1, single dose; 2, single dose at 3× concentration; 3, triple therapy; 4, triple therapy with washing. (■) Expected bioavailability; (▨) observed bioavailability.

whereas only 20 μl was applied in the single-dose equivalent. Although the acetone evaporated quickly, 60 μl of acetone was administered in three serial doses of 20 μl over 24 hr, possibly producing a "solvent-vehicle" effect. Conceivably, each application of acetone and drug instantaneously rediluted or redissolved any dry hydrocortisone present at the skin surface or within the stratum corneum, possibly increasing mobilization and bioavailability of material to the skin. Such a dilution effect was unlikely during single-dose treatments. Hence, during multiple dosing the dilution effect may have increased the bioavailability of hydrocortisone in the skin, resulting in an observed increase in percutaneous absorption.

Surprisingly, removal of the drug between washes during multiple-application treatments did not significantly affect absorption. As mentioned under Results, it was anticipated that during the 24 hr after the initial application of hydrocortisone to the skin, more hydrocortisone was available to the skin surface during treatment 3, where it was not removed, than during treatment 4, where it was removed by washing prior to application of the subsequent dose. Table II indicates that 16% more drug was removed from the skin during treatment 4 than in treatment 3. Washing the site may have rediluted the drug at the skin surface or rehydrated the skin itself, thereby increasing bioavailability and absorption (7). This effect was reported in other *in vivo* experiments (2,7); nevertheless, it has yet to be proven by experiments specifically designed to do so.

Some of the differences between these data and previous *in vivo* studies can be attributed to differences in experimental design. Unlike previous experiments, test material was not applied to an "open" skin site; rather, protective propylene (Hilltop) chambers were employed to improve mass balance. The chambers utilized during this study were modified to protect the site from physical disturbance while rendering the site essentially unoccluded. It had been verified by Bucks *et al.* that these chambers allowed more movement of water and air about the skin than ordinary polypropylene (Hilltop) chambers (4). Consequently, it has not been determined if these polypropylene chambers specifically influence absorption differently than open skin sites except in the context of transepidermal water loss and mass balance after application of various para-substituted phenols (4). The chamber was also employed to control the application and removal schedule of the radiolabeled material better, unlike previous clinical trials by Bucks *et al.*, where washing and accidental removal by rubbing of hydrocortisone became an attribute of each volunteer's routine (3). Hence some differences in results between the studies mentioned may have been caused by increased physical containment of hydrocortisone; the chamber has been shown to improve mass balance through retention of drugs ordinarily lost through exfoliation during open studies (4). Finally, due to the 0.2-μm pore size of the containment membrane, one may speculate that sufficient transepidermal water loss and eccrine sweat produced at the application site during exposure to the drug may have significantly promoted skin hydration, thereby increasing mobilization and bioavailability of the drug in the skin (6).

Previous long-term *in vivo* trials by Wester *et al.* in the rhesus monkey attribute an observed increase in hydrocorti-

Table IV. Comparison of Hydrocortisone Delivery Between Treatments in Terms of Average Percentage of Applied Dose and Mass Absorbed per Surface Area

	1 × 13.33 μg/cm²	1 × 40.00 μg/cm²	3 × 13.33 μg/cm²; no wash	3 × 13.33 μg/cm²; wash
		Percentage of applied dose[a]		
1 × 13.33 μg/cm²	—	*0.579*	**0.006**	**0.016**
1 × 40.00 μg/cm²	—	—	**0.023**	**0.026**
3 × 13.33 μg/cm²; no wash	—	—	—	*0.059*
3 × 13.33 μg/cm²; wash	—	—	—	—
		Mass absorbed per cm²[a]		
1 × 13.33 μg/cm²	—	*0.055*	**0.022**	**0.027**
1 × 40.00 μg/cm²	—	—	**0.023**	**0.026**
3 × 13.33 μg/cm²; no wash	—	—	—	*0.059*
3 × 13.33 μg/cm²; wash	—	—	—	—

[a] The data are two-tailed, paired, P values determined by Student's t test between the treatments compared. Italicized values signify no significant differences between treatments. Boldface values denote significant differences ($P < 0.05$) between treatments.

sone bioavailability to epidermal thinning due to repeated application of cold hydrocortisone over the first 8 days of the study (2). This effect is unlikely to be the reason for the observed increase in hydrocortisone absorption during multiple dosing in this study since significantly less material (three 13.33 μg/cm² applications maximum) was applied.

CONCLUSION

There is little information available on the most effective topical corticosteroid dosing regimen regarding the number of skin applications in a day. Multiple applications for an ambulatory patient with a readily accessible skin site are a common practice. However, for hospitalized patients, patients with less accessible skin sites, or patients with occluded skin sites, a single daily dose may be more practical. Along with cost, a single daily dose may be the most efficient if therapy is not compromised. Earlier animal studies using the same vehicle, acetone, suggest that drug bioavailability is not changed with increased daily application (2). This study suggests that triple therapy in man may have some advantage (Fig. 1). If increased bioavailability is desired, then multiple-application therapy may be the answer. Our data suggest the possibility that increased bioavailability is related to reapplication of vehicle; hence, a case may be made for increasing hydrocortisone bioavailability merely by applying serial doses of acetone to an ample, previously applied single dose of hydrocortisone at the skin surface. Such an experiment would verify if the solvent-vehicle effect was the only component by which multiple application of hydrocortisone in acetone increased its bioavailability in human skin. Unfortunately, observations from such trials would bear little to no relevance to the questions which are the clinical bases for investigation of hydrocortisone multiple dosing. Our observations and previous experiments also offer some suggestion that soap and water wash enhanced skin absorption (2,3). This may also be related to the solvent action of water and the detergent. Whether bioavailability could be increased with other solvents or other nonsolvent formulations is not known. Hence further investigation of this matter would be best directed at determining hydrocortisone bioavailability from multiple-application dosing with conventional vehicle formulations.

These pharmacokinetic studies revealing differences between man and the rhesus monkey suggest the complexity of the issues involved in dosing corticosteroids topically. In addition to future experiments outlined above, we must examine other corticosteroids of varying physicochemical properties and their effects on normal versus abnormal skin.

ACKNOWLEDGMENTS

We gratefully acknowledge W. L. Gore and Associates, Inc., who generously supplied our project with their 0.2-μm PTFE membranes as gifts. We would also like to thank Mrs. Miyako Li for her technical assistance.

REFERENCES

1. R. C. Wester, P. K. Noonan, and H. I. Maibach. Percutaneous absorption of hydrocortisone increases significantly with long term administration: In vivo studies in the rhesus monkey. *Arch. Dermatol.* 116:186–188 (1980).
2. R. C. Wester, P. K. Noonan, and H. I. Maibach. Frequency of application on percutaneous absorption of hydrocortisone. *Arch. Dermatol.* 113:620–622 (1977).
3. D. A. W. Bucks, H. I. Maibach, and R. H. Guy. Percutaneous absorption of steroids: Effect of repeated application. *J. Pharm. Sci.* 74(12):1337–1339 (1985).
4. D. A. W. Bucks, H. I. Maibach, and R. H. Guy. Mass balance and dose accountability in percutaneous absorption studies: Development of a nonocclusive application system. *Pharm. Res.* 5:313–315 (1988).
5. R. C. Wester and H. I. Maibach. Relationship of topical dose and percutaneous absorption in rhesus monkey and man. *J. Invest. Dermatol.* 67:518–520 (1976).
6. R. C. Wester and H. I. Maibach. Rhesus monkey as an animal model for percutaneous absorption. In H. Maibach (ed.), *Animal Models in Dermatology,* Churchill Livingstone, London and New York, 1975, pp. 133–137.
7. R. C. Wester and H. I. Maibach. In H. Maibach and B. Bronaugh (eds.), *Percutaneous Absorption,* Marcell Dekker, New York, 1985, pp. 327–333.