Percutaneous Absorption of Steroids: Effect of Repeated Application

To the Editor:

Most percutaneous absorption experiments have employed a single administration of the compound under investigation. However, the relevant clinical situation usually involves multiple application of drug to the same skin site. Equally, systemic toxic effects can result from repeated cutaneous exposure to occupational and environmental chemicals and, again, the typical case involves repetitive contact of the hazard with a common skin site.

Despite the obvious clinical and occupational relevance of understanding pharmacokinetics following multiple topical application to the skin, there have been few reports of such studies. Roberts and Horlock[1] investigated the effect of repeated skin application on the percutaneous absorption of salicylic acid in rats and obtained results which were at least partly consistent with the keratolytic effect of the drug. Wester and co-workers[2] considered skin penetration of ^{14}C-labeled hydrocortisone after short- and long-term administration, using the rhesus monkey. The results showed that the level of hydrocortisone absorption significantly increased during long-term administration; it was suggested that this observation was the result of hydrocortisone-induced alteration of the penetration barrier. Most recently, the percutaneous absorption of malathion, following repeated administration to humans, has been measured.[3] Long-term absorption was not significantly different from short-term behavior, implying that single dose application data are perhaps relevant for predicting the toxic potential of malathion after repeated cutaneous exposure.

The present study explored further the relationship between percutaneous absorption and frequency of application. The penetration of three steroids, hydrocortisone, testosterone, and estradiol, after repeated administration to human subjects, has been evaluated and compared with that observed after a single topical dose.

Percutaneous absorption was determined by measurement of the percentage of applied radioactivity excreted into urine for 7 d following application of a known amount of radiolabeled compound to the skin. Five normal healthy male volunteers formed each of the three study groups. Carbon-14 labeled hydrocortisone, testosterone, or estradiol in an acetone vehicle was applied to 28 cm^2 of ventral forearm skin. The surface concentration of the chemical was 4 $\mu g/cm^2$. The site of application was not occluded. Each radiolabeled chemical dose consisted of 1 μCi of carbon-14.

Radiolabeled compound was applied on day 1 and day 8, and unlabeled material was applied on days 2 through 7 and 9 through 14 to the same site at the same surface concentration. This schedule of applications is not necessarily optimal. The selection was made principally on the basis of following the protocol employed in an earlier multidose study with malathion.[3] Ideally: (*a*) the period between carbon-14 applications should ensure complete washout of the prior radiolabeled dose, and (*b*) additional 'hot' dose/'cold' dose sequences should be incorporated so that normal skin turnover time is encompassed within the study period. However, it was believed that volunteer convenience and compliance would most easily accommodate a 2-week study; hence, for the purpose of this preliminary investigation, the above-mentioned format was pursued.

The site of application was washed every day ~1 h prior to the next administration. To simulate a clinical situation, the subjects were instructed to follow their normal morning washing procedures. Thus, while a representative in vivo situation was followed, information concerning the level of chemical removed by washing was lost, and mass balance determinations were not possible.

Urine was collected daily and analyzed for carbon-14. Fourteen milliliters of PCS scintillation cocktail (Amersham Corp.) was added to 5 mL of each urine sample. Urine samples were prepared in triplicate. To determine the extent of quenching, a [^{14}C]toluene internal standard was added to one of the triplicate sample preparations. Daily urinary carbon-14 excretion values were corrected for retention of radioactivity in the body, and elimination of radioactive material by other routes, using data from the intravenous administration of ^{14}C-labeled steroid.[4]

Tables I, II, and III present the percutaneous absorption data for hydrocortisone, testosterone, and estradiol, respectively. Total absorption of the compounds resulting from the first and eighth applications is illustrated in Table IV; for each steroid, a paired *t* test revealed no significant difference ($p > 0.05$) in carbon-14 absorption between these two doses.

In Table IV, we also compare the total absorption of the three steroids resulting from the two ^{14}C-labeled applications with the corresponding single-dose data of Feldmann and Maibach.[4] In this earlier work, exactly comparable procedures were followed except, of course, that chemical was applied only once on day 1. For each steroid, an unpaired *t* test was used to assess whether absorption following a single application differed from that following either the first or eighth dose of the multi-application regimen. At $p > 0.05$, with the exception of a barely significant difference for testosterone (single dose compared to the first dose of the repeated administration schedule), no statistically identifiable difference in absorption was apparent. Thus, for these steroids at the dose level employed, it appears that percutaneous absorption following daily, repeated application can be adequately predicted from a single application experiment.

Some caveats to this conclusion should be stated at this point. First, the subject numbers are not large and the variability is characteristic of a normal in vivo percutaneous absorption experiment. Second, the washing procedures were "typical" and deliberately uncontrolled. Third, the "ideal" criterion of complete washout of carbon-14 prior to the second labeled dose is not met. However, hydrocortisone, for example, shows that 10% of the 7-day excretion total is eliminated on the seventh day postdose of both the first and second carbon-14 doses. This equivalency supports the contention of close coincidence between the levels of absorption following the two radioactive administrations. Therefore, while accepting that complete experimental control has been sacrificed to maintain the relevancy of the study, the data point, nevertheless, to the acceptance of the null hypothesis: skin penetration of steroids does not change significantly with multiple dosing under the administration schedule described.

This conclusion is not consistent with the hydrocortisone multiple-dose absorption study of Wester and co-workers conducted in rhesus monkey.[2] In this previous investigation,

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Table I—Hydrocortisone Percutaneous Penetration[a]

					Hydrocortisone, First Carbon-14 Dose						Total Dose, %
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	1	1	1	1	2	3	4	5	6	7	
Subject 1	0.03	0.05	0.10	0.27	0.41	0.58	0.50	0.44	0.47	0.29	3.1
2	0.06	0.03	0.05	0.20	0.50	0.46	0.50	0.37	0.47	0.27	2.9
3	0.08	0.10	0.27	0.16	0.38	0.29	0.07	0.28	0.11	0.14	1.9
4	0.05	0.12	0.04	0.16	0.36	0.52	0.53	0.44	0.49	0.42	3.1
5	0.14	0.02	0.03	0.12	0.29	0.28	0.26	0.28	0.26	0.14	1.8
Average	0.07	0.07	0.10	0.18	0.39	0.43	0.37	0.36	0.36	0.25	2.6[b]
					Hydrocortisone, Second Carbon-14 Dose						
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	8	8	8	8	9	10	11	12	13	14	
Subject 1	0.12	0.12	0.12	0.28	0.87	0.75	0.71	0.66	0.66	0.60	4.9
2	0.11	0.26	0.17	0.48	0.84	0.59	0.52	0.48	0.33	0.25	4.0
3	0.11	0.09	0.07	0.11	0.15	0.40	0.21	0.21	0.08	0.13	1.6
4	0.29	0.12	0.09	0.18	0.78	0.77	0.54	0.38	0.46	0.50	4.1
5	0.14	0.07	0.06	0.13	0.35	0.38	0.34	0.25	0.33	0.23	2.3
Average	0.15	0.13	0.10	0.24	0.59	0.58	0.46	0.39	0.37	0.34	3.4[c]

[a] Data are expressed as percent of applied dose absorbed per time interval. [b] SD = 0.7. [c] SD = 1.4.

Table II—Testosterone Percutaneous Penetration[a]

					Testosterone, First Carbon-14 Dose						Total Dose, %
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	1	1	1	1	2	3	4	5	6	7	
Subject 1	0.78	0.17	1.8	15	3.6	0.67	0.39	0.07	0.27	0.22	23
2	3.1	5.7	9.0	2.6	2.4	1.0	0.32	0.38	0.33	0.42	25
3	1.6	2.3	1.9	0.66	3.3	4.4	1.9	1.4	0.86	0.29	18
4	0.15	0.85	1.4	4.6	10.0	4.4	2.6	1.6	1.0	0.86	28
5	0.05	0.79	0.92	5.1	3.2	0.57	0.76	0.24	0.23	0.22	12
Average	1.1	2.0	3.0	5.7	4.6	2.2	1.2	0.73	0.54	0.40	21[b]
					Testosterone, Second Carbon-14 Dose						
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	8	8	8	8	9	10	11	12	13	14	
Subject 1	0.11	0.27	9.4	5.5	0.26	0.22	1.1	1.2	0.15	0.05	18
2	1.2	1.4	3.9	5.5	2.1	1.7	0.79	0.27	0.19	0.51	17
3	0.05	0.04	0.06	0.07	1.9	0.54	1.4	1.1	0.86	0.66	6.7
4	0.15	0.15	0.74	5.6	11.0	4.5	2.8	3.1	1.6	0.75	30
5	0.04	0.28	0.07	5.3	1.5	3.3	2.1	1.0	0.67	0.67	15
Average	0.31	0.42	2.8	4.4	3.3	2.1	1.6	1.3	0.68	0.53	17[c]

[a] Data expressed as percent of applied dose absorbed per time interval. [b] SD = 6.2. [c] SD = 8.4.

significant enhancement in absorption was observed for the second ^{14}C-labeled dose (i.e., the eighth chemical dose). It was suggested that repeated application of the steroid was perhaps causing epidermal thinning during the experiment such that the barrier on day 8 was markedly lower than on day 1, thereby allowing greater penetration of the drug. An alternative explanation implicating a reservoir effect has also been suggested.[5] Why the human in vivo results presented here differ from the earlier work is not clear. Some procedural differences exist: (*a*) in the monkey study, cold material was not administered on days 9 through 14 and the site of application was not washed during this period; (*b*) the washing procedures in this study were different from those used in the animal model experiments; and (*c*) no interference with the application site (e.g., by rubbing or contact with clothing) occurred in the monkey study. The relative importance of these differences remains unknown.

The results of the work presented here are consistent with the multi-application data of Wester and co-workers[3] using malathion. In this case, no methodological differences can be

Table III—Estradiol Percutaneous Penetration[a]

					Estradiol, First Carbon-14 Dose						Total Dose, %
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	1	1	1	1	2	3	4	5	6	7	
Subject 1	0.12	0.31	0.56	0.56	0.45	1.0	1.4	1.6	0.84	0.37	7.2
2	0.23	0.48	1.3	1.2	0.33	2.7	3.5	1.7	1.2	0.78	13
3	0.22	0.06	0.01	0.26	1.9	2.1	2.6	0.82	0.83	0.43	9.2
4	0.20	0.12	0.05	0.21	1.3	4.6	2.0	0.72	0.97	0.75	11
5	0.04	0.06	0.10	0.33	2.1	4.7	0.21	0.53	0.41	0.59	9.1
Average	0.16	0.20	0.39	0.51	1.2	3.0	2.0	1.1	0.85	0.58	9.9[b]
					Estradiol, Second Carbon-14 Dose						
Interval, h	4	4	4	12	24	24	24	24	24	24	
Day	8	8	8	8	9	10	11	12	13	14	
Subject 1	0.54	0.19	0.91	0.36	0.42	0.41	1.2	0.88	0.98	1.0	6.9
2	0.46	0.88	1.0	3.0	2.2	3.8	1.0	2.9	2.0	0.79	18
3	0.12	0.19	0.14	0.36	2.2	0.54	0.98	1.2	0.43	0.26	6.4
4	0.10	0.19	0.36	0.21	1.8	2.7	1.7	1.3	1.8	1.4	12
5	0.15	0.08	0.10	0.30	2.4	2.9	1.9	0.70	0.32	2.3	11
Average	0.27	0.30	0.50	0.85	1.8	2.1	1.4	1.4	1.1	1.1	11[c]

[a] Data expressed as percent of applied dose absorbed per time interval. [b] SD = 2.3. [c] SD = 4.7.

Table IV—Percutaneous Absorption of Hydrocortisone, Testosterone, and Estradiol in Humans

	Dose Absorbed, % (Mean ± SD)		
	Hydrocortisone	Testosterone	Estradiol
Single dose[a]	1.9 ± 1.6	13.2 ± 3.0	10.6 ± 4.9
First dose[b]	2.6 ± 0.7	22.1 ± 6.9	9.9 ± 2.3
Eighth dose[b]	3.4 ± 1.4	20.2 ± 6.8	10.8 ± 4.7

[a] Data from ref. 4. [b] Data from this study.

identified except that malathion was topically applied as the neat chemical at a concentration much higher (5 mg/cm^2) than that used in the steroid work. Penetration of the pesticide was relatively poor; there was no significant difference between absorption following the first and eighth chemical doses.

In summary, we conclude that many questions pertaining to the effect of repeated application on percutaneous absorption in humans remain unanswered. As yet, the multiple application of the chemicals studied has not revealed any obvious changes in the skin barrier or reservoir as a function of dose. This remains at odds with the reported animal model experiments.[1,2] Because of the therapeutic and toxicological relevance of the repeated dose situation, resolution of the problems indicated by this research is needed. It may be suggested that human experimentation (with larger subject populations), in which more careful control of the environment of the application site is maintained (such that the entire disposition of the administered drug can be determined), represents a logical starting point for the next sequence of investigations.

References and Notes

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