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Percutaneous absorption of triamcinolone acetonide from creams with and without Azone® in humans in vivo

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Summary

A clinical study was performed in which 0.05% triamcinolone acetonide cream (TA), containing tracer amounts of ^{3}H-labelled steroid, was applied once on the volar aspect of the right forearm for 12 h under occlusion. No Azone was incorporated in this cream. After a wash-out period, repeated dosing of TAZ, a similar cream containing 0.05% triamcinolone acetonide and 1.6% Azone, was performed on days 4–7 on the left forearm for 12 h per day under occlusion. The TAZ-cream contained tracer amounts of the ^{3}H-labelled steroid on days 4 and 7. Absorption, distribution, and elimination of triamcinolone acetonide were followed by measuring the amounts of ^{3}H-steroid-derived radioactivity in plasma, tape strippings, urine, and feces. Residual analysis was performed as well. During the whole study-period 6.7 ± 0.3% (mean ± S.D.) of the total applied radioactivity penetrated through the skin. The first application of TAZ resulted in a 3.2-times higher percutaneous absorption of steroid than the single application of TA, indicating an increased rate of absorption of triamcinolone acetonide in the presence of Azone. After multiple dosing of TAZ, absorption further increased to 6.8 times that of a single TA dose. Nevertheless, systemic levels of triamcinolone acetonide remained very low. Tape stripping revealed that reservoir formation of steroid was less pronounced after multiple dosing of TAZ relative to single dosing of TA, and higher levels of steroid were found in the deeper layers of the stratum corneum, immediately after removal of the last TAZ-dose. This paper reports the first evidence that Azone acts as a penetration enhancer in vivo in human skin when dosed in a therapeutic formulation.

Introduction

Triamcinolone acetonide is one of the most frequently prescribed topical glucocorticosteroids for anti-inflammatory and anti-mitotic purposes. Before exerting its pharmacological action in the target cells in the dermis, triamcinolone acetonide will have to penetrate the main barrier for percutaneous absorption, the stratum corneum.

The efficacy of triamcinolone acetonide may be improved by increasing its percutaneous absorption. A rational way to facilitate the penetration process of drugs through the skin barrier is the use of penetration enhancers. One of the most promising compounds in this respect is 1-dodecylazacycloheptan-2-one (Azone®, Nelson Research, Irvine, CA, U.S.A.) which has demonstrated to be

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a very useful, nontoxic, nonirritating penetration enhancer for the percutaneous absorption of triamcinolone acetonide (Stoughton, 1982a; Chow et al., 1987). The in vivo activity of triamcinolone acetonide was shown to be effective in the vasoconstriction assay in humans at 3 and 8% Azone, the lower concentration giving a higher efficacy of the drug (Stoughton 1982b; Stoughton and McClure, 1983). In an in vitro experiment simultaneous administration of the two compounds resulted in an increased rate and extent of triamcinolone acetonide absorption. The in vitro enhancing effect of the penetration of triamcinolone acetonide was dependent on the concentration of Azone and skin type and was found to be optimal at 2–5% v/v for human cadaver skin (Chow et al., 1987), which is commensurate with the already mentioned in vivo results, and at 2–3% for hairless mouse skin (Chow et al., 1984).

Recently, a new cream, TAZ (Nelson Research), containing 0.05% triamcinolone acetonide and 1.6% Azone was developed. In order to assess a possible enhancement of percutaneous absorption of triamcinolone acetonide from this cream due to the inclusion of the penetration enhancer Azone, a clinical study was performed in three male healthy volunteers, in which single and multiple dermal applications of TAZ were compared to a single application of a comparable triamcinolone acetonide cream without Azone (TA). Unfortunately, a full comparison between single and multiple dosing of Azone-containing and Azone-free triamcinolone acetonide creams could not be performed for medical ethical reasons.

The study was carried out with radioactive material ([^{3}H]-triamcinolone acetonide) as no method was available to follow unlabelled parent compound or metabolites in the biomatrix at the low concentrations at which they occur.

Materials and Methods

Materials

Creams and chemicals

The three creams used in this study are listed in Table 1. All substances other than Azone and triamcinolone acetonide were present in the creams in the same amounts. In the case of Azone containing creams, Azone was substituted for water.

The radioactive compound ([1,2,4(n)-^{3}H]triamcinolone acetonide, Amersham, Buckinghamshire, U.K.) in the therapeutic formulation was extracted from the creams using methanol and analysed by isocratic HPLC to be of 98.4% purity.

The creams were manufactured by Nelson Research. Before and after administration of the cream, the amount of triamcinolone acetonide was determined by HPLC. The amounts retrieved are listed in Table 1 as well. The specific activity of the triamcinolone acetonide in the therapeutic formulations was 3.06 and 2.44 GBq/mmol for cream A and B, respectively. This corresponds to 408.5 and 358.9 kBq/100 mg cream, respectively. Cream C was formulated with non-radioactive triamcinolone acetonide.

All other materials were reagent grade, obtained commercially and used without further purification.

TABLE 1

Theoretical and actual concentrations of cream constituents, before and after the study (mean ± SE)

Cream code	Triamcinolone acetonide (% w/w)			Radioactivity (kBq/100 mg)	Azone (% w/w)
	Theor.	Before	After		
A (TA)	0.05	0.0580 ± 0.0009	0.0568 ± 0.0010	408.5 ± 6.2	–
B (TAZ)	0.05	0.0638 ± 0.0020	0.0579 ± 0.0010	358.9 ± 17.9	1.6
C (TAZ)	0.05	0.0567 ± 0.0002	0.0549 ± 0.0001	–	1.6

Methods

Study design

Three male healthy volunteers participated in this study. Prior to the start each volunteer was subjected to a standard physical examination and a complete medical history was taken. All volunteers gave their written informed consent. The study was performed in accordance with FDA's Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) regulations and guidelines. A schematic outline of the study is given in Fig. 1.

The volar aspect of both forearms was shaven 3 days prior to the start of the study. At day 1 a template of adhesive foil (Ensure-it™, Deseret Medical, Inc., Sandy, UT, U.S.A.) of 10 × 14 cm, leaving an area of 4 × 6 cm of the skin uncovered, was applied to the volar aspect of the right forearm. Approx. 100 mg of cream A (TA, see Table 1) was applied to the skin and spread across the entire area using a metal spatula. The application area was then covered with a plastic foil which was taped to the underlying template in order to obtain occlusive conditions. The exact amount of radioactivity applied to the skin was calculated from the weight of the spatula before and after administration of the dose and the radioactivity per unit weight of cream.

After 12 h, during which the volunteers were seated in a chair, the template and cover foil were removed and the dosage remaining on the skin was recovered by wiping the skin with gauze pads and rinsing the application area with ethanol sponging six times. The gauze pads, template, cover foil and rinse sponges were saved for radioactivity analysis.

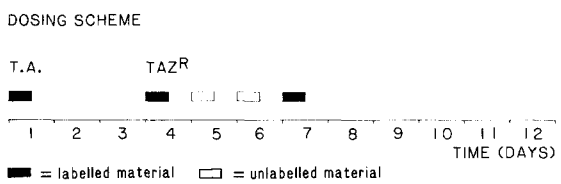

Fig. 1. Schematic study outline. Each box represents a dose of 100 mg cream, containing tracer amounts of [^{3}H]triamcinolone acetonide (0.37 MBq, black boxes) or no tracer (open boxes). Dosages applied on days 4–7 contained 1.6% Azone (TAZ cream).

On day 4, approx. 100 mg of cream B (TAZ, see Table 1) was applied to the volar aspect of the left forearm. All study conditions such as application area, time, occlusion and removal of the dose were as described above.

On days 5 and 6, approx. 100 mg of cream C (TAZ, see Table 1) was applied to the same area of the left forearm as the day before; study conditions were all the same. Repeated dosing on the same application area was achieved by marking the boundaries of the treated area.

On day 7, approx. 100 mg of cream B was applied to the same area of the left forearm as treated before without alterations in study conditions.

Urine, blood, and feces were collected at regular intervals up to 5 days after removal of the last dose, the total study period being 12 days. Blood sampling was performed in the cubital veins of both arms simultaneously in order to compare the concentrations just behind the application area (ipsilateral) and the systemic levels (contralateral). All blood samples were collected in heparin containing glass-tubes and centrifuged in order to obtain plasma.

Tape stripping, which removes a layer of stratum corneum, was performed at 1, 20, and 44 h after removal of the single triamcinolone acetonide dose without Azone on the right arm, i.e. at days 1, 2, and 3, respectively, according to the method described previously (Wiechers et al., 1987). After multiple dosing of TAZ, the tape stripping procedure was performed on the left arm at 1, 20, and 44 h after removal of the fourth TAZ application, i.e. on days 7, 8, and 9, respectively. The strips were placed in counting vials in groups of four. A total of 28 strips was taken per occasion. Stripping was done in such a way that after the third occasion more than 90% of the application area had been stripped of its stratum corneum (Wiechers et al., 1990).

The volunteers were observed for complaints and for signs and symptoms of any treatment-related effect throughout the whole study period and blood pressure, heart rate, respiration rate, and body temperature were measured twice a day. After having completed the study all volunteers again passed the standard physical examination.

Analytical procedures

Radioactive material was extracted from the swabs, gauze pads, template and cover foil using methanol, in which triamcinolone acetonide is very soluble. A weighed aliquot (approx. 1 ml) was pipetted in polyethylene counting vials and analysed for radioactivity after the addition of 3 ml of the scintillation cocktail RiaLuma (Lumac, Landgraaf, The Netherlands). The sum of the amounts recovered in these samples represented the external recovery.

A 1 ml aliquot of plasma was mixed with 4 ml RiaLuma in polyethylene counting vials. The vials were vigorously shaken by hand and counted by liquid scintillation spectrometry.

After determination of the volume of each urine fraction, a 2 ml aliquot was mixed with 12 ml RiaLuma in glass scintillation vials, and counted after vigorous shaking by hand.

An aliquot of lyophilized and homogenized feces was combusted in a Tri-Carb sample oxidizer (Packard, Downers Grove, IL, U.S.A.) in a stream of oxygen and nitrogen. The resulting radioactive carbon dioxide was absorbed in 10 ml Carbosorb, after which the latter was mixed with 11 ml Permafluor as scintillation cocktail (both from Packard Technologies, Groningen, The Netherlands). Proton exchange from tritium labelled triamcinolone acetonide and/or metabolites may result in tritiated water, which could be lost during freeze-drying. In order to check this phenomenon as well as possible co-sublimation of radioactive material during lyophilization, sublimated material was collected after de-icing and analyzed for radioactivity.

To the tapes of the stripping procedure, 18 ml RiaLuma was added in glass scintillation vials. The vials were vigorously shaken for 16 h and then analysed.

Radioactivity was measured on a Packard Minaxi Tri-Carb B4450 Liquid Scintillation Spectrometer (Packard Technologies, Groningen, The Netherlands) or a Beckman LS 1800 Liquid Scintillation Spectrometer (Beckman, Irvine, CA, U.S.A.) for 10 min or a statistical accuracy of 0.5%. Counting efficiencies were determined for each sample individually by assessing the H-number or the Spectral Index of External Standard, when using the Beckman or Packard counter, respectively. Subsequently, the outcomes were converted to disintegrations per second (Bq). No differences were observed when comparing the two scintillation systems.

Results

The recovery of [^{3}H]triamcinolone acetonide-derived radioactivity in the various samples was calculated as the amount of radioactivity present in the samples relative to the total dose applied. A survey of the recoveries is presented in Table 2. The total percutaneous absorption of triamcinolone acetonide over the whole study period, based

TABLE 2

Recovery values of [^{3}H]triamcinolone acetonide-derived radioactivity in various samples, expressed as the percentage of the total dose applied

Sample type	Volunteer			
	01	02	03	Mean ± S.D.
External recovery	74.06	66.50	67.69	69.4 ± 4.1
Plasma	< 0.01	< 0.01	< 0.01	< 0.01
Urine	4.25	3.76	3.12	3.7 ± 0.6
Feces	2.05	1.99	2.64	2.2 ± 0.4
Tape strippings	0.81	0.93	0.68	0.8 ± 0.1
Total				
recovery (%)	81.17	73.18	74.13	76.2 ± 4.4
dose applied (MBq)	1.107	1.071	1.093	1.09 ± 0.02

TABLE 3

External recovery of [^{3}H]triamcinolone acetonide-derived radioactivity, expressed as the percentage of the corresponding daily dose

Day	Volunteer			
	01	02	03	Mean ± S.D.
1	76.30	77.98	80.79	78.4 ± 2.3
4	88.99	78.14	68.98	78.7 ± 10.0
7	57.88	43.35	50.55	50.6 ± 7.3

on the amounts found in urine, feces, and tape strippings, added up to 6.74 ± 0.34% (mean ± S.D.) of the applied dose. Due to the slow elimination of triamcinolone acetonide, its absorption after each individual dose could not be calculated. Table 3 lists the external recoveries of [^{3}H]-triamcinolone acetonide-derived radioactivity expressed as the percentage of the corresponding daily dose. These data suggest that larger amounts are being absorbed after multiple dosing of the Azone-containing cream as smaller amounts of triamcinolone-derived radioactivity could be retrieved from the skin.

The distribution of [^{3}H]triamcinolone acetonide-derived radioactivity in the stratum corneum as determined by the tape stripping method is shown in a three-dimensional plot (Fig. 2). The tape numbers shown on the *X*-axis are indicative of the depth in the skin, the *Y*-axis shows the amount of radioactive material retrieved in the strips and the changes of these in time are shown along the *Z*-axis. The total amounts of radioactivity retrieved at the various days are given in Table 4. From this table and from Fig. 2, it can be concluded that there is less triamcinolone acetonide-derived radioactivity present in the skin after multiple dosing of TAZ than following a single dosing

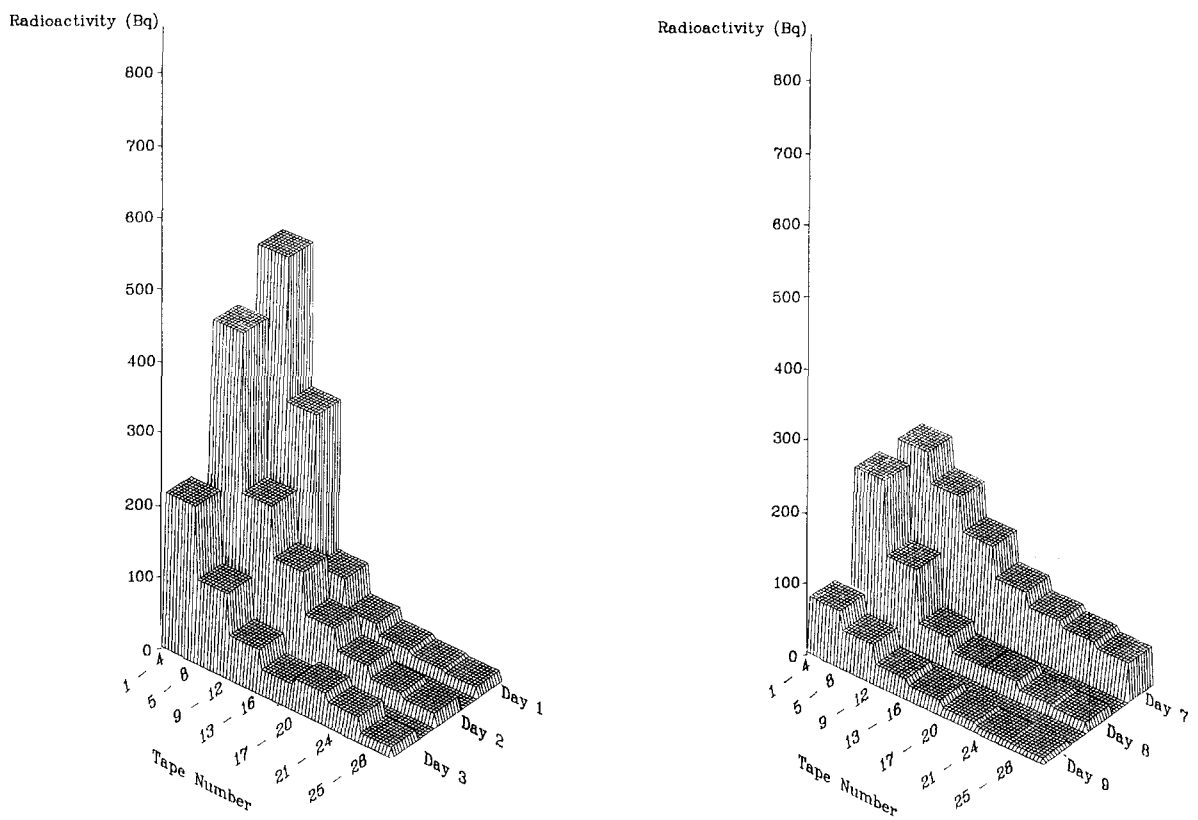

Fig. 2. Distribution of [^{3}H]triamcinolone acetonide-derived radioactivity in the stratum corneum in the absence (A) and presence (B) of the penetration enhancer Azone.

TABLE 4

Total amounts of [^{3}H]triamcinolone acetonide-derived radioactivity in the stratum corneum on three consecutive days following a single dose without Azone (A), and after multiple dosing with Azone (B) (data in Bq)

	Day of stripping	Volunteer			
		01	02	03	Mean ± S.D.
A	1	3312	3149	2655	3039 ± 342
	2	1437	2137	888	1487 ± 626
	3	1161	1203	555	973 ± 363
B	7	2551	1918	2931	2467 ± 512
	8	291	1266	293	617 ± 562
	9	187	279	159	208 ± 63
Totals	*A*	5910	6489	4089	5496 ± 1252
	B	3029	3463	3383	3292 ± 231
Ratio	*B/A*	0.51	0.53	0.83	0.62 ± 0.18

of TA, especially in the upper layers, indicating that reservoir formation of triamcinolone acetonide is less pronounced after multiple dosing in the presence of the penetration enhancer Azone, relative to single dosing of the steroid.

The ipsi- and contralateral plasma levels of [^{3}H]triamcinolone acetonide-derived radioactivity are shown in Fig. 3. The somewhat higher contralateral plasma level at 24 h was observed in only one volunteer, the other two having levels very close to the detection limit. This single observation is therefore believed to be an outlier and explains the relatively large standard error of the mean of the value at 24 h.

The urinary excretion accounted for 62.4 ± 7.2% (mean ± S.D.) of the total excretion. The urinary excretion profile of [^{3}H]triamcinolone acetonide-derived radioactivity is shown in Fig. 4 and indicates that excretion is complete within 4 days after the last application. The urinary elimination half-lives were determined from the slopes of the regression lines of a semilogarithmic plot of the excretion rate vs midpoint time of urine collection, and were found to be 11.4 ± 2.0 h (mean ± S.D.) following single dosing of TA at day 1, and 11.8 ± 0.4 h following multiple application of TAZ at day 7.

Fecal excretion accounted for 37.6 ± 7.2% (mean ± S.D.) of the total excretion. The amounts of radioactivity retrieved in the feces are listed in Table 5. The sublimated material of the lyophilization process contained 3.4% of the total amount retrieved. A general correction was made for this quantity, called Rest. Table 5 suggests that the fecal excretion is not entirely complete for all three volunteers.

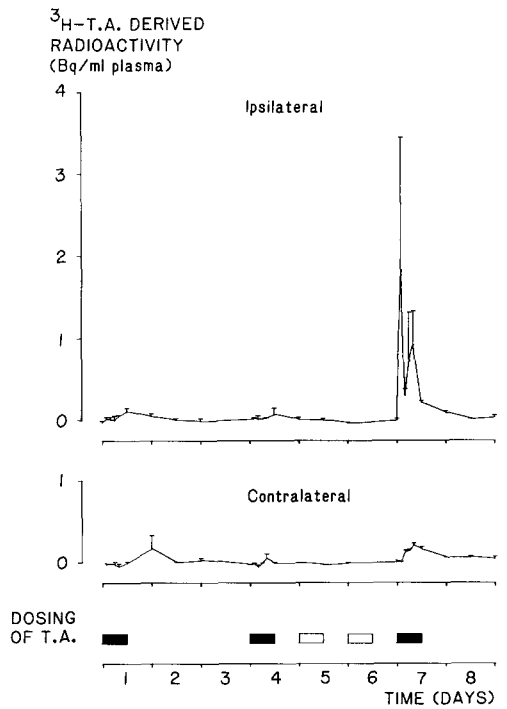

Fig. 3. [^{3}H]triamcinolone acetonide-derived radioactivity in ipsi- and contralateral plasma samples (mean ± S.E.). The dosing scheme of labelled and unlabelled triamcinolone acetonide is shown as well.

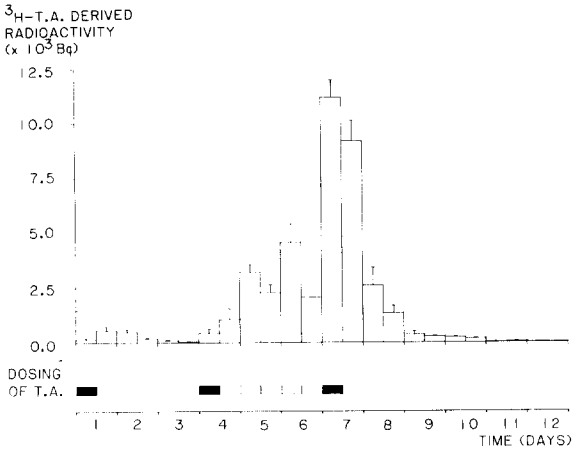

Fig. 4. Amounts of [^{3}H]triamcinolone acetonide derived radioactivity in the urine per interval vs time (mean ± S.E.). The dosing scheme of labelled and unlabelled triamcinolone acetonide is shown as well.

Discussion

Theoretically, the amounts of ^{3}H radioactivity that are not externally recovered should have been absorbed through the skin. This implies that in this study some 30% of the applied steroid would have been absorbed (see Table 2). Yet the amounts retrieved in the various biosamples are substantially smaller at 6.7 ± 0.3% (mean ± S.D.). Urinary excretion was complete as can be seen in Fig. 4. Although the data in Table 5 suggest that fecal excretion was not complete, the amounts of radioactivity found in the feces are rather small in comparison to the dose applied. This indicates that no substantial amounts of radioactivity would have been retrieved if additional feces samples had been available. Moreover, the urine/feces excretion ratio of 62.4/37.6 in this study closely corresponds to that of 70/30 as observed in earlier studies (Helmy, 1987). Accumulation of triamcinolone acetonide in the stratum corneum in a so-called reservoir has been shown to occur (Vickers, 1963). However, both application areas were completely stripped to their full depth during this study, thereby making it unlikely that the remainder of the absorbed radioactivity would still be present in the skin. The deviation in the total recovery value is therefore conceivably due to the rather low doses of radioactivity that had to be given for safety reasons and the large number of samples involved in the assessment of the total recovery. This may have resulted in a lower accuracy (many samples below the limit of determination) and an accumulation of errors. Furthermore, it should be realized that this study was a dual label experiment in which Azone was labelled with ^{14}C so that the penetration enhancer could be followed as well (Wiechers et al., 1990). It is known that the presence of ^{14}C will reduce the precision of the ^{3}H determination and vice versa (Herberg, 1964).

The impact of the incorporation of Azone on the extent of percutaneous absorption of triamcinolone acetonide cannot be derived from the urinary and fecal excretion of radioactivity as can be seen from Fig. 4 in which the urinary excretion on day 7 is likely to represent material from the radioactive doses on days 4 and 7. Table 3, however, indicates on the basis of the external recoveries of

TABLE 5

[^{3}H]Triamcinolone acetonide-derived radioactivity retrieved in feces (mean ± S.D.) and sublimated water after lyophilization (rest)

Volunteer	Time (h:min)	Radioactivity (Bq)	(Σ Bq)
1	85:17	1362.2± 455.0	
	134:07	2063.2± 104.5	
	240:21	9198.7± 368.8	
	274:33	9322.4± 682.8	
	Rest	738.8	22685.3
2	38:12	391.7± 65.9	
	85:17	2977.6± 429.5	
	158.32	5317.2± 341.0	
	218:09	11909.1± 1028.4	
	Rest	693.3	21288.9
3	27:43	651.5± 573.7	
	62:20	204.3± 72.4	
	85:20	715.2± 19.7	
	109:43	529.0± 44.2	
	132:58	829.8± 53.2	
	158:25	1363.7± 428.6	
	173:46	2465.7± 284.9	
	193:37	6811.2± 206.9	
	196:03	13345.4± 1654.6	
	271:53	1008.0± 36.1	
	Rest	940.0	28863.8

the individual doses that the amounts of triamcinolone acetonide absorbed from a therapeutic formulation without and with Azone are of the same magnitude after a single application, but that steroid absorption increases particularly after multiple dosing of TAZ. The latter will then result in higher levels of drug in the viable skin.

This can also be observed in Fig. 2. After the single application of TA at day 1, 1 h after removal of the dose most radioactive material is found in the outermost layers of the stratum corneum. At the second and third day, considerable amounts of radioactive material are still present in the outermost layers (Fig. 2A). This is the so-called reservoir effect, widely known for topically applied steroids (Vickers, 1963). At 1 h after removal of the fourth TAZ dose, also a considerable amount of radioactive material is present but the drug has penetrated more deeply into the stratum corneum. At the second and third day after removal of the fourth TAZ dose, much less activity can be found in the stratum corneum (Fig. 2B) than after the single dose of TA. Table 4 also indicates that smaller amounts of radioactivity are present in the stratum corneum after multiple dosing of TAZ compared to single dosing of TA. In this respect, Azone appears to be different from other penetration enhancers such as dimethyl sulphoxide (DMSO) (Stoughton, 1965), *N*-methyl-2-pyrrolidone (NMP), and dimethylformamide (DMF) (Barry et al., 1984), that are known to induce reservoir formation of steroids in the skin, based on the potent dissolving characteristics of these promotors. As the latter diffuse more quickly through human skin than the steroid, the drug precipitates in the stratum corneum and a reservoir is formed (Barry, 1983). In this dual label study, however, it could be demonstrated that triamcinolone acetonide-derived radioactivity penetrated more quickly through the stratum corneum than Azone-derived radioactivity did. As Azone does not induce the reservoir formation of triamcinolone acetonide, this may indicate that this penetration enhancer exerts its action directly on the barrier function of the skin rather than by dissolving the steroid. Different mechanisms of action have indeed been proposed for DMSO, NMP and DMF on the one hand and Azone on the other hand (Barry, 1987).

This increased extent and rate of percutaneous absorption of triamcinolone acetonide following multiple dosing of TAZ is also visible in the plasma values. Both ipsi- and contralateral blood samples were drawn in this study. The ipsilateral plasma levels are expected to be higher, reflecting drug concentrations near the site of absorption, whereas the contralateral levels, representing the systemic concentration will be lower due to distribution (Karim, 1983). An increased extent of percutaneous absorption would then result in higher systemic levels, whereas an increased rate would correspond to an increased difference between ipsi- and contralateral levels.

Contralateral plasma levels (Fig. 3) have increased after multiple dosing of TAZ on day 7 as compared to day 1, indicating increased penetration of the steroid, but do not exceed 0.3 Bq/ml, i.e. 0.05 ng/ml (metabolism not accounted for). Again, a single dose of TAZ, however, did not significantly raise the systemic plasma levels (day 4 vs day 1).

Likewise, after the first application of TAZ at day 4, the difference between ipsi- and contralateral levels is comparable to that after the single application of TA at day 1, while after multiple dosing of TAZ this difference has increased considerably. These plasma data prove that the percutaneous absorption of triamcinolone acetonide especially increases after multiple dosing of TAZ but that a single dose of TAZ has little measurable effect on rate and extent of steroid absorption.

If more steroid has been absorbed in the presence of Azone, this should be reflected in the excretion profiles. Unfortunately, the feces data have to be ignored as the retrieved radioactivity cannot be assigned to one of the three labelled doses. To a much smaller extent, the same is true for the urinary excretion data. As can be seen from the urinary excretion plot (Fig. 4), only small quantities of the amount dosed at day 1 penetrated through human skin, and the urinary excretion appears virtually complete at the end of the third day. After dosing of radioactive triamcinolone acetonide on day 4, but now in the presence of Azone, the amounts excreted in the urine during days 4–6 are much larger than on days 1–3, indicating increased absorption even after a single dose of TAZ. The alternating excretion pattern ob-

served during days 4–6 may be explained by the repeated occlusion of the application area during days 5 and 6, which is known to result in an accelerated depletion of reservoir in the epidermis into the body (McKenzie and Stoughton, 1962; Barry, 1983).

To quantitate the increase in percutaneous absorption of triamcinolone acetonide after single and multiple dosing of TAZ, enhancement factors are introduced. In in vitro skin permeation experiments, the enhancement factor is expressed as the quotient of the steady-state flux of penetrant in the presence of the enhancer over that in the absence of the enhancer (Barry, 1987; Chien, 1987). In this in vivo experiment, the steady-state flux cannot be determined. Therefore, the enhancement factors have been calculated as the ratio of the total amount of radioactivity excreted after administration of TAZ over that excreted after administration of TA. In this way, the single dose enhancement factor (SDEF) can be expressed as the percentage of the dose excreted at days 4–6 over days 1–3; the multiple dose enhancement factor (MDEF) as days 7–9 over days 4–6, and finally, the total enhancement factor (TEF) as days 7–9 over 1–3. Yet as the radioactivity excreted during day 7 cannot be definitely assigned to one of the TAZ formulations dosed at day 4 or 7, an exact calculation of the enhancement factors will not be possible. Nevertheless, by assuming that urinary excretion of the dose from day 4 is complete at the end of day 6, an estimate of the enhancement factors can be derived from the amounts retrieved in the urine and the stratum corneum during different time intervals. The latter has to be corrected for the percentage excreted in the feces.

The outcomes of these calculations are listed in Table 6, and indicate that the incorporation of Azone enhances the percutaneous absorption of triamcinolone acetonide after a single dose by a factor 3.2 ± 0.8 (mean ± S.D.), while multiple dosing of TAZ further increases the extent of percutaneous absorption by a factor 2.1 ± 0.4. The total enhancement factor was found to be 6.8 ± 2.8.

Table 6 shows another interesting feature: A decreasing relative standard deviation can be seen for the total percentages excreted in the urine after a single application of TAZ, and even more

TABLE 6

Amounts of tritium excreted in the urine and retrieved in the stratum corneum, supposed to be excreted in the urine, expressed as the percentage of the corresponding dose applied, and the single, multiple dose and total enhancement factor (see text for details)

Parameter	Volunteer				
	01	02	03	Mean ± S.D.	R.S.D.
Urinary excretion					
Days 1–3	0.338	0.653	0.201	0.40 ± 0.23	58.3
4–6	4.249	4.486	3.039	3.93 ± 0.78	19.8
7–9	8.282	6.929	6.727	7.13 ± 0.85	11.6
Stratum corneum[a]					
Days 1–3	1.034	1.156	0.542	0.91 ± 0.33	35.7
7–9	0.547	0.635	0.540	0.57 ± 0.05	9.3
Total					
Days 1–3	1.372	1.809	0.743	1.31 ± 0.54	41.0
4–6	4.249	4.486	3.039	3.93 ± 0.78	19.8
7–9	8.829	7.564	7.267	7.89 ± 0.83	10.5
Enhancement factors					
SDEF	3.10	2.48	4.09	3.2 ± 0.8	25.2
MDEF	2.08	1.69	2.39	2.1 ± 0.4	17.1
TEF	6.44	4.18	9.78	6.8 ± 2.8	41.4

[a]Calculated as % of dose in stratum corneum multiplied by the fraction urinary excretion of the volunteer in question.

so after multiple dosing of TAZ. These findings may suggest that Azone decreases intersubject variability in percutaneous absorption.

The incorporation of Azone had no effect on the urinary excretion rate of triamcinolone-derived radioactivity. It can therefore be concluded that the percutaneous absorption of triamcinolone acetonide is not the rate-limiting step in the clearance of ^{3}H radioactivity. Otherwise, the increased absorption rate due to Azone and multiple dosing conditions would have resulted in increased elimination and thus shorter elimination halflives.

All results in this clinical study suggest that relative to single dosing of TA, both the extent and rate of percutaneous absorption of triamcinolone acetonide have increased after multiple dosing of TAZ. Although the effect on the absorption of steroid following a single dose of TAZ was less pronounced, it is clear from the urinary excretion data that the extent of absorption has increased. Because the time of application was 12 h on both occasions, this implies an increased rate of absorption as well. This increase can only be ascribed to the incorporation of Azone into the therapeutic formulation as all other experimental conditions were identical. The increase after multiple dosing, however, may not entirely be due to the incorporation of Azone alone but may be partially evoked by the multiple dosing of the steroid itself and/or the repeated occlusion of the application area. Additional clinical studies are in progress to elucidate these phenomena in more detail.

Summarizing, this paper reports the first evidence that Azone acts as a penetration enhancer in human skin in vivo when dosed from a therapeutic formulation.

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