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Report

Percutaneous Absorption and Elimination of the Penetration Enhancer Azone in Humans

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The percutaneous absorption and elimination of Azone, a new penetration enhancer, were investigated in humans. The distribution and accumulation of Azone in the skin were studied by means of tape stripping. These studies reveal that pure Azone is poorly absorbed. Furthermore, what little Azone is absorbed appears to be rapidly cleared from the circulation by the kidneys. In order to explain the urinary excretion profile, the formation of at least one metabolite is suggested. No accumulation of Azone in the skin was observed.

KEY WORDS: Azone; percutaneous absorption; penetration enhancer; elimination; humans; skin; *in vivo* studies.

INTRODUCTION

Topical administration of drugs avoids many of the problems that arise with oral and the more invasive methods of drug delivery. Unfortunately, the skin provides an efficient transport barrier, thus rendering administration of drugs for systemic purposes rather difficult. Compounds such as dimethylsulfoxide (DMSO), propylene glycol, urea, and pyrrolidones have proven to be of some use in enhancing the percutaneous absorption of chemical agents but have limitations as well. Azone (1-dodecylazacycloheptan-2-one) is an agent that greatly enhances the percutaneous absorption of hydrophylic compounds and, to a lesser extent, hydrophobic compounds (1).

Several studies on the penetration enhancing effects of Azone have been performed recently, giving promising results for a number of therapeutic agents (2–5), but nothing is known about the penetration of Azone itself. Azone is a colorless, odorless, and chemically inert, oily liquid, insoluble in water but freely soluble in many organic solvents. The structural formula of Azone is given in Fig. 1. Its toxicity is very low and comparable to that observed with nutritional compounds (1). Moreover, it is a nonirritant to human skin, even when applied in an undiluted form (6).

In order to assess further its safety and to get some insight into its pharmacokinetic properties, the percutaneous absorption and elimination of pure Azone in humans were studied. This was done by applying ^{14}C-labeled Azone to the skin of the forearm and monitoring the radioactivity in blood, feces, and urine over 5 days. The possibility of reservoir formation in the stratum corneum was investigated by tape stripping.

So far, most experiments with Azone indicate a maximum enhancement of penetration of therapeutic agents at 1–4% and very little enhancement at 15% and above. In our experiment, however, 100% Azone was applied in order to obtain the absorption and elimination parameters of Azone as such. These values can then be used as a reference when studying Azone at lower doses and in dosage forms, with or without therapeutic agents.

MATERIALS AND METHODS

Materials

1-Dodecylazacycloheptan-2-one (1-^{14}C-dodecyl), specific activity 303 GBq/mol, was obtained from Atomlight, North Billerica, Mass. The radiochemical purity was determined by thin-layer chromatography (TLC) to be at least 97.5% [TLC plates, Merck Silica Gel; solvent chloroform:methanol (40:1) unsaturated chamber]. Azone was kindly supplied by Nelson Research (Irvine, Calif.). All other materials were reagent grade and obtained commercially.

Preparation of the Dosage

The dosage was prepared by mixing a methanolic radio-

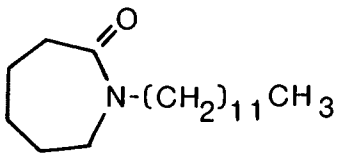

Fig. 1. Structural formula of Azone (1-dodecylazacycloheptan-2-one).

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active solution with nonlabeled Azone and evaporating the methanol under a stream of nitrogen at room temperature. The dosage consisted of pure Azone, containing 100 μCi ^{14}C-Azone/100 μl.

Study Design

Three male healthy volunteers participated in this study. Prior to the start of the study each volunteer was subjected to a standard physical examination program and a complete medical history was taken. All volunteers gave their written informed consent.

The study consisted of a single administration of 100 μl of Azone, containing 100 μCi of ^{14}C-labeled Azone. The volar aspect of the left forearm was shaven, and a template of adhesive foil (Ensure-it, Parke, Davis & Company, Sandy, Utah; 10 × 14 cm) was applied to the arm to define a 4 × 4-cm area of uncovered skin.

The dose was applied to the skin with a syringe and spread across the entire area with a metal spatula. When Azone leaked outside this area onto the template, it was wiped off with a gauze pad which was saved for analysis. After 4 hr, during which time the arm was left uncovered while resting on a bench, the remaining dosage was removed by wiping the skin with gauze pads. The boundaries of the treated area were marked and the template was removed. The skin was thoroughly rinsed with ethanol sponging six times. The spatula, gauze pads, template, and rinse sponges were saved for radioactivity analysis.

Urine, blood, and feces were collected at regular intervals during 5 additional days. Blood samples consisted of 10 ml whole blood, collected in heparin-containing Venoject tubes. All urine, blood and feces samples were stored at −20°C until analysis.

During the experiment the blood pressure, heart rate, respiration rate, and body temperature were measured twice a day.

Before and after having completed the experiment all subjects passed a physical examination and clinical chemistry including hematology, blood chemistry, and urine analysis.

Skin Sampling

Tape stripping, which removes a layer of stratum corneum, was performed at 5, 24, and 48 hr after application of the dosage, i.e., 1, 20, and 44 hr, respectively, after removal of the dose. This was done by means of commercial cellophane translucent adhesive tape of 9-mm width, made by the 3M Company (Leiden, The Netherlands). A total of 28 strips, approximately 6 cm in length, was adhered and removed sequentially to the same transverse portion of the treatment site.

At each application, the numbered strip was firmly rubbed in place to achieve thorough adherence and then removed after about 3 sec. Four sequential strips were placed in one scintillation counting vial and saved for analysis.

Separate parallel transverse portions of the treatment site were evaluated at 3 successive days.

When tape stripping became painful, because the viable epidermis was reached, 24 strippings instead of 28 were performed.

Analytical Procedures

Radioactive material in the swabs, gauze pads, template, and spatula was repeatedly extracted with the scintillation cocktail Plasmasol (Packard, Groningen, The Netherlands), and the extracts were counted, if necessary after dilution, by liquid scintillation spectroscopy. The sum of the amounts recovered in these samples represented the external recovery.

To each set of four tapes from the tape stripping procedure, 16 ml of Plasmasol was added. The vials were vigorously shaken and counted after 1 day. The radioactive concentration in urine samples was determined by mixing 1 ml of the urine sample with 4 ml of Pico-Fluor 30 (Packard), then vigorously shaking and counting the solution.

Blood samples of 1 ml were solubilized with 1 ml NCS (Amersham, Houten, The Netherlands) and decolorized with hydrogen peroxide (30%). To the colorless samples glacial acetic acid (0.5 ml) was added to reduce chemiluminescence, followed by 15 ml Plasmasol. Counting was done after waiting for at least 1 day. Feces were lyophilized and homogenized. Then the samples were counted after combustion in a Packard Tri-Carb sample oxidizer.

RESULTS

The recovery of ^{14}C-Azone-derived radioactivity is given in Table I. The material recovered externally from the skin appeared to be unchanged Azone.

The most important observation in this study is the low absorption of Azone in humans. This can be concluded from the high external recovery values as well as the low amounts retrieved in blood, urine, and feces (see Table I). Excretion in the urine and in the feces was nearly complete after 5 days, as exemplified in Fig. 2. More than 94% of the excreted radioactivity was found in the urine.

The external recovery of volunteer 3 is lower than those of the other volunteers. Yet this does not necessarily mean that this volunteer absorbed more Azone since excretion data in urine and feces and the radioactivity in the blood and the skin are quite similar to those in the other two volunteers. An explanation of this low value may be an unnoticed loss of Azone during the application period.

Furthermore, it may well be that the amounts absorbed by volunteers 1 and 2 are smaller than the 7.5 or 2.3%, respectively, that are not externally recovered because it is extremely difficult to recover all radioactivity from the skin.

The absolute amounts of radioactivity recovered in the skin and the distribution thereof are depicted in Fig. 3. It can be seen that there is little accumulation of radioactivity in the stratum corneum, which rapidly disappears in time.

Table I. Recovery of ^{14}C-Azone-Derived Radioactivity Expressed as a Percentage of the Dose Applied

Volunteer No.	External recovery	Tape stripping	Blood	Urinary excretion	Fecal excretion
1	92.5	0.031	0.000	0.089	0.003
2	97.7	0.028	0.000	0.114	0.003
3	64.8	0.024	0.000	0.193	0.010

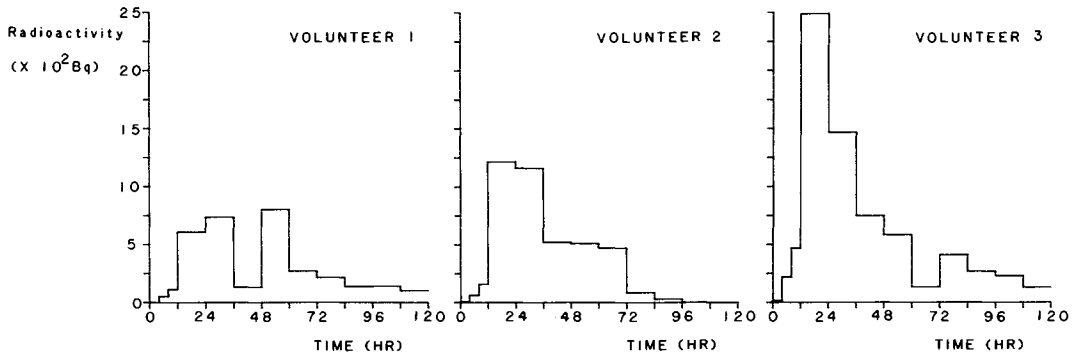

Fig. 2. Urinary excretion profile of ^{14}C-Azone-derived radioactivity.

Because of the low absorption of Azone, no radioactivity could be detected in blood. All values were below the limit of accurate determination. The latter was about 4 Bq/ml, which corresponds to 1×10^{-4}% of the dose applied. In feces and urine, however, radioactivity could be detected in low amounts. Data on urinary and fecal excretion are given in Tables II and III, respectively. The urinary excretion profile showed an irregular pattern (see Fig. 2). It should be noted, however, that the dips in the curves of volunteer 1 at 36–48 hr and of volunteer 3 at 60–72 hr corresponded with rather low amounts of urine voided during these periods (98 and 146 ml, respectively). During the first 4 hr of the experiment there is virtually no excretion in the urine, whereas maximum excretion occurs in the 12 to 48-hr interval. Within 48 hr, 50–75% of the total excreted amount is recovered. Little radioactivity was excreted during the fifth day of the experiment (0–7.3%).

No adverse reactions related to Azone occurred. There was no significant influence of Azone on blood pressure, heart rate, respiration rate, or body temperature. The posttreatment blood chemistry, hematology, and urine analysis did not show significant changes.

DISCUSSION

The percutaneous absorption of Azone is small. It remains predominantly on the skin and the small percentage that becomes absorbed is present to a large extent in the upper layer, the stratum corneum, as can be seen in Fig. 3. This layer is the main barrier for percutaneous absorption (7). When using full-thickness skin of hairless rats in an *in vitro* permeation technique involving diffusion cells, it was found for 5-fluorouracil that Azone affects mainly the stratum corneum, while the permeation of 5-fluorouracil through the dermis is hardly influenced (3,5). The stratum corneum is also known as a place where reservoir formation can occur. Such a phenomenon might explain the prolonged activity of Azone: up to 120 hr in full-thickness skin *in vitro* after a single application (2). The tape-stripping data clearly indicate, however, that a reservoir formation does not occur, as little Azone is present at 20 and 44 hr after the removal of the dose (see Fig. 3), indicating a difference in pharmacokinetic behavior of Azone in *in vitro* and *in vivo* experiments. Assuming an identical distribution of nonlabeled and ^{14}C-labeled Azone, the concentrations of Azone and metabolites (expressed in nanomoles) in the skin at 1, 20, and 44 hr after

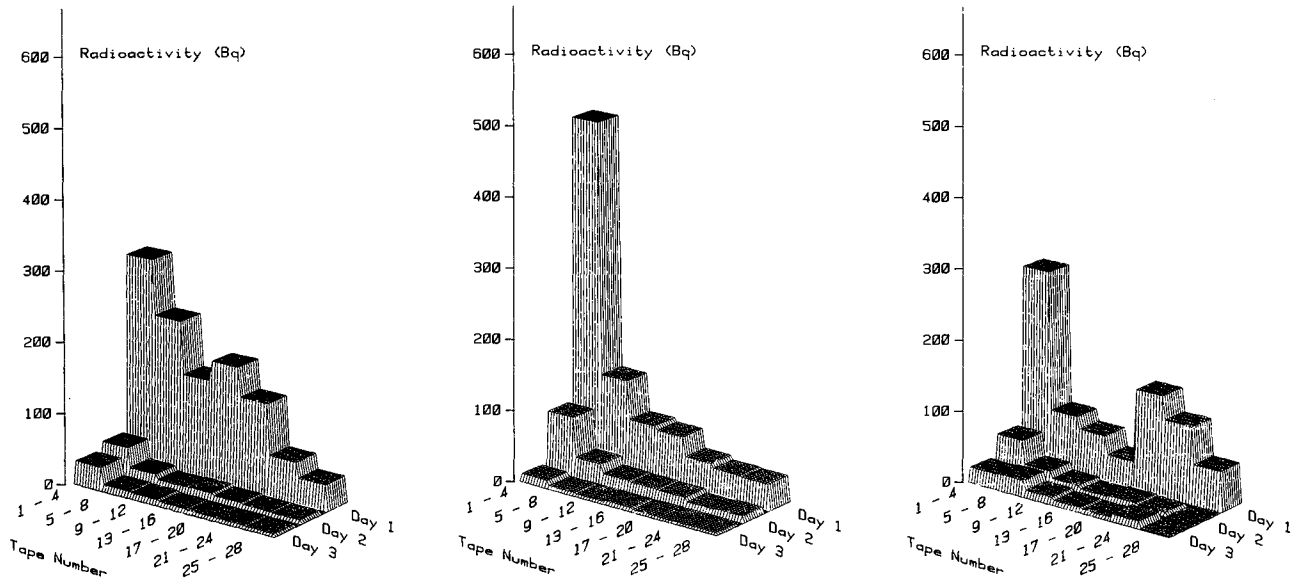

Fig. 3. Distribution of and elimination from the skin of ^{14}C-Azone-derived radioactivity, obtained by tape stripping. Left, middle, and right are the results of volunteers 1, 2, and 3, respectively. For details, see text.

Table II. Urinary Excretion of ^{14}C-Azone-Derived Radioactivity (Data in Bq)

Time (hr)	Volunteer No. 1	2	3
0–4	0.0	6.3	14.0
4–8	52.3	62.9	216.5
8–12	111.8	157.2	467.4
12–24	609.5	1214.7	2488.8
24–36	736.7	1158.7	1465.7
36–48	129.2	516.8	745.6
48–60	805.2	508.0	580.6
60–72	266.3	469.4	127.5
72–84	211.4	82.0	409.7
84–96	134.0	26.4	261.8
96–108	140.7	0.0	224.4
108–120	99.4	0.0	125.0
Total	3296.5	4202.4	7127.0

removal of the dose can be calculated. These concentrations are given in Table IV, indicating that after 44 hr the concentrations are down to 10–20 nmol/cm^2. It should be noted, however, that metabolism is not accounted for. If one then considers that Wotton *et al.* still found an Azone effect 120 hr after a single application of a formulation containing 1% Azone (2), and assuming comparable kinetics in *in vitro* and *in vivo* situations, one may conclude that at the latter time the amounts of Azone found in the stratum corneum must have been extremely small.

In Table V the disappearance rate of radioactivity from the skin is expressed as the half-life. However, the accuracy of the values for 44 hr is not very high because of the low levels of radioactivity, so that the resulting half-lifes for the second day should be interpreted with care.

A semilogarithmic plot of the urinary excretion rate versus midpoint time of urine collection gave an estimation of pharmacokinetic parameters (see Table VI). Mathematically the urinary excretion profile can be described by

Table III. Fecal Excretion of ^{14}C-Azone-Derived Radioactivity

Volunteer No.	Sample No.	time (hr:min)	Radioactivity (Bq)
1	1	31:50	12.0
	2	53:30	37.4
	3	89:10	52.3
Total			101.7
2	1	7:33	11.0
	2	26:33	14.8
	3	77:58	35.1
	4	98:23	59.4
Total			120.3
3	1	10:20	119.3
	2	25:10	87.2
	3	47:05	82.6
	4	69:35	33.9
	5	72:35	46.0
	6	97:35	27.9
Total			396.9

Table IV. Concentration of Azone and/or Metabolites (nmol/cm^2) Calculated from ^{14}C-Azone-Derived Radioactivity in the Skin at 1, 20, and 44 hr After Removal of the Dose

Volunteer No.	Concentration (nmol/cm^2) 1 hr	20 hr	44 hr
1	318	29	20
2	266	51	8
3	230	29	22

$$C_t = B \cdot e^{k_{el}} - A \cdot e^{k_a}$$
$$t^{el}_{1/2} = (\ln 2)/k_{el}$$
$$t^{a}_{1/2} = (\ln 2)/k_a$$

in which

A and B = constants,
C_t = the concentration at time t,
k_{el} = the elimination rate constant,
k_a = the absorption rate constant,
$t^{a}_{1/2}$ = the half-life of absorption into the systemic circulation, and
$t^{el}_{1/2}$ = the half-life of elimination from the systemic circulation.

The rising and declining part of the curve (α and β phase, respectively) correspond to the absorption and elimination phase into and from the systemic circulation, respectively, provided the elimination is the rate-limiting step, and vice versa if absorption is the rate-limiting step. Assuming the latter to be the case, the absorption half-life would be approximately 24 hr (see Table VI). Then the concentration in the skin at the time of the second tape stripping would have to be of the order of 60% of that initially found in the first tape strippings. As this is not the case (see Table V), absorption into the systemic circulation does not seem to be the rate-limiting step.

Yet comparison of the elimination half-life of radioactivity in the human skin during the first day with the urinary half-life for the α phase (see Table VI) shows that the rate at which radioactivity disappears from the skin during the first day is of the same magnitude as the appearance rate of radioactivity in the urine, indicating the disappearance of radioactivity from the human skin to be the rate-limiting step. As these statements seem to contradict each other, a third phenomenon may be involved in explaining our findings. The much longer half-life of the declining part of the urinary excretion plot may be explained by a redistribution of ab-

Table V. Amounts of Radioactivity Recovered in the Tape Strippings, Expressed as the Percentage Relative to the Amount Initially Found; Disappearance Half-Life During the First and Second Day

Volunteer No.	Relative concentration at hr 1	20	44	Half-life (hr) during hr 1–20	20–44
1	100.0	9.2	6.2	5.5	42
2	100.0	19.1	3.0	8.0	9.0
3	100.0	12.7	9.4	6.4	55

Table VI. Half-Lives Estimated from the Urinary Excretion Rate Plot and the Tape-Stripping Data (Data as Hours)

Volunteer No.	Urinary excretion		Tape stripping	
	α phase	β phase	Day 1	Day 2
1	5.4	34.7	5.5	42
2	11.5	11.6	8.0	9
3	5.8	25.0	6.4	55
Mean ± SD	7.6 ± 3.4	23.8 ± 11.6	6.6 ± 1.3	35.3 ± 23.7

sorbed Azone in the body or, more likely, the formation of one or more metabolites of which the formation or the excretion is slower than the absorption of radioactivity into the systemic circulation. The formation of a metabolite(s), which is then the rate-limiting step of the elimination phase, is very likely because the radioactivity was excreted mainly via the urine, whereas Azone itself is very lipophilic and therefore anticipated to appear—at least partly—in the feces when excreted as the parent compound.

It should be noted that this study was performed with undiluted Azone. As Azone is usually applied at low concentrations in percutaneous formulations, its body burden in those cases will be even lower than in this study. These findings, in combination with the fact that Azone is nontoxic to animals (1,6,8), indicate that Azone should be quite safe for human use.

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