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Percutaneous absorption, metabolism, and elimination of the penetration enhancer Azone in humans after prolonged application under occlusion

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Summary

Azone was applied undiluted to an extended area of the forearm and left in place for 12 h under occlusion in 3 volunteers. Azone was found to be absorbed in very low amounts ($0.419 \pm 0.259\%$ of the applied dose) and accumulation in the body did not occur. As compared to a previous study, the new application conditions gave an increase in amount and percentage absorbed, but a decrease in flux. Absorbed material was excreted rapidly and almost exclusively through the urine after extensive metabolism to at least 3 rather polar compounds. These findings and the absence of treatment-related side-effects in the 3 volunteers suggest azone to be safe for human use when topically applied under occlusion.

Introduction

Azone (1-dodecylazacycloheptan-2-one, Fig. 1) is an agent that greatly enhances the percutaneous absorption of drugs (Stoughton, 1982; Stoughton and McClure, 1983). It is a colorless, odorless and pharmacologically inert oily liquid, insoluble in water, but freely soluble in many organic solvents. In animals its toxicity is very low and comparable to that observed with nutritional compounds (Stoughton and McClure, 1983). Also, it is non-irritant to human skin, even in undiluted form (Stoughton, 1982).

In a previous study using ^{14}C-labelled material, we evaluated the percutaneous absorption and elimination of azone in humans after applying the undiluted substance to a relatively small area of the forearm for 4 h under non-occlusive conditions. Absorption was found to be very low and elimination occurred almost exclusively through the kidneys, probably in the form of one or more metabolites (Wiechers et al., 1987). The absolute amounts in the urine, however, were too low to conclusively demonstrate the presence of these matabolites.

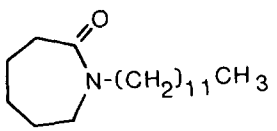

Fig. 1. Structure of azone (1-dodecylazacycloheptan-2-one).

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Therefore, in the present study it was tried to increase the percutaneous absorption of azone by enlarging the application area, by prolonging the exposure time and by applying the dosage under occlusion. ^{14}C-labelled azone had to be used since no analytical method is available to follow the unlabelled parent compound and/or its metabolites.

Materials and Methods

Chemicals

1-Dodecylazacycloheptan-2-one ([1-^{14}C]dodecyl), spec. act. 303 GBq/mol, was obtained from Atomlight, North Billerica, MA, U.S.A. The radiochemical purity was determined by thin-layer chromatography (TLC) to be 96.8% (TLC plates, Merck Silica Gel; solvent chloroform : methanol (40 : 1), unsaturated chamber) and by isocratic high-performance liquid chromatography (HPLC) to be 95.4% (the eluent being methanol/phosphate buffer 0.01 M, pH 6.8 (90/10), and using the system described below).

Labelled an unlabelled azone were kindly supplied by Nelson Research (Irvine, CA, U.S.A.). All other materials were reagent grade and obtained commercially.

Preparation of the dosage

The dosage was prepared by mixing a methanolic radioactive solution with non-labelled azone and evaporating the methanol under a stream of nitrogen at room temperature. The dosage consisted of pure azone, containing 2.75 MBq [^{14}C]azone/50 μl. The calculated specific activity of the dosage prepared this way was 17.1 GBq/mol.

Study design

Three male healthy volunteers who had participated in a previous study (Wiechers et al., 1987) were asked to enroll in this study. Prior to the start 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 50 μl of pure azone, containing ca. 2.75 MBq of ^{14}C tracer. The volar aspect of the left forearm was shaven, and a template of adhesive foil (Ensure-it, Parke, Davis Co., Sandy, UT, U.S.A.) of 10×14 cm was applied to the arm to define a 4×6 cm area of uncovered skin. The dose was applied to the skin with a syringe and spread across the entire area outlined by the boundaries of the template with a metal spatula. After 30 min the application area was covered by a plastic foil which was taped to the underlying template. The cover foil was left in place for 12 h, during which time the volunteers were seated in a chair. The exact amount applied was calculated from the weight of the syringe used for the administration of the dose before and after administration and the radioactivity per unit weight of the dosage, corrected for the amount of radioactivity retrieved on the metal spatula.

After 12 h, the template was 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 spatula, template, gauze pads, and rinse sponges were saved for radioactivity analysis.

The volunteers were observed for complaints and for signs and symptoms of any treatment-related effect during 5 additional days and these were recorded at the time of blood sampling.

Blood pressure, heart rate, respiration rate and body temperature were measured twice a day. Urine, blood and feces were collected at regular intervals during the whole study period. Blood samples consisted of two times 5 ml whole blood, and were collected in heparin-containing plastic tubes. One of these samples was centrifugated to obtain plasma. All urine, whole blood, plasma and feces were stored at $-20\,^{\circ}$C until analysis.

After having completed the study, all volunteers again passed the standard physical examination.

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 spectrometry. The sum of the amounts recovered in these samples represented the external recovery.

To 1 ml urine 4 ml Pico-Fluor 30 (Packard) was added, and counted after vigorous shaking.

Blood samples of 0.25 ml were solubilized using 2 ml of a mixture of Soluene-350 (Packard) and 2-propanol (1 : 1), and decolorized with hydrogen peroxide (30%). To the colorless samples, 10 ml of the chemiluminescence-reducing scintillation cocktail Hionic-Fluor (Packard) was added and the mixtures were counted after vigorous shaking.

Plasma samples were counted after thoroughly mixing 0.5 ml plasma with 10 ml Plasmasol.

Feces were lyophilized and homogenized. Samples of ca. 450 mg dry weight were counted after combustion in a Packard Tri-Carb sample oxidizer.

Radioactivity was measured by a Packard Minaxi Tri-Carb B4450 Liquid Scintillation Spectrometer (Packard Instruments, Groningen, The Netherlands) or a Beckman LS1800 Liquid Scintillation Spectrometer (Beckman, Irvine, CA, U.S.A.) for 5 min or a statistical accuracy of 0.5%.

Metabolic profiling of urine samples

Urine fractions were lyophilized and homogenized. To an aliquot of ca. 275 mg dry material 2.0 ml methanol was added and the mixture was centrifuged after vigorous shaking. The supernatant of the methanolic extract, containing 82.8 $\pm$ 2.3% of the total radioactivity of the sample, was evaporated under a stream of nitrogen after which 2.0 ml of water was added to the residue.

This 2.0 ml sample was injected into an HPLC system consisting of two Waters M510 HPLC pumps (Millipore, Etten-Leur, The Netherlands), controlled by an Adalab data acquisition/control system (Interactive Microware, Inc., State College, PA, U.S.A.). A concave gradient as depicted in Fig. 2, from 100% phosphate buffer 0.01 M pH 6.8 to 100% methanol in 60 min, followed by a methanol flush of 15 min, and a flow rate of 1.0 ml/min, was capable of eluting components of vastly different polarity within an acceptable time. The column (150 $\times$ 4.6 mm, i.d.) was made of stainless steel, and packed with Nucleosil 7 C18 (Macherey-Nagel, Düren, F.R.G.). Chemicals were

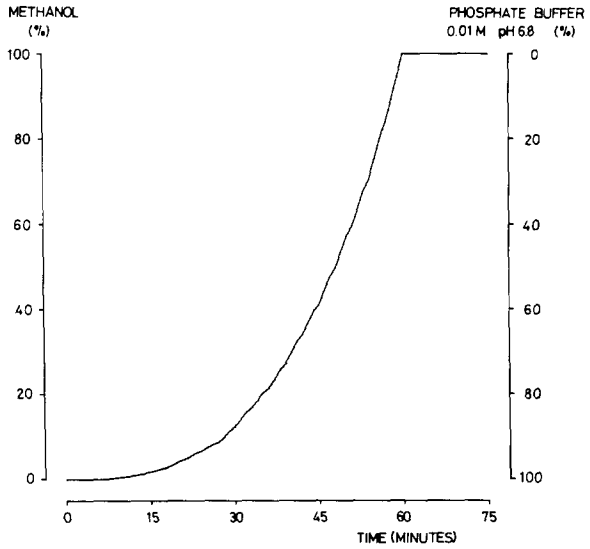

Fig. 2. Eluent composition during the chromatographic analysis of the urine samples.

of analytical grade and dissolved in bidistilled water. A 2 ml injection loop was used in a Rheodyne injection valve model 7125 (Inacom, Veenendaal, The Netherlands). Effluent fractions of 1 min were collected and after vigorous shaking with 4 ml RiaLuma (Lumac, Landgraaf, The Netherlands) counted on the Packard Minaxi B4450 Liquid Scintillation Spectrometer for 5 min or a statistical accuracy of 0.5%.

Results and Discussion

The percutaneous absorption of azone measured as the percentage of applied radioactivity excreted over 5.5 days in urine and feces, was very low as indicated in Table 1. Complementary evidence in provided by the high external recovery.

The determination of the total recovery was an important objective in this study because of the relatively low value obtained in a previous study for one of the volunteers (Wiechers et al., 1987). Therefore, the same volunteers were asked to participate in this study. This time, the total recovery almost equalled 100% for all volunteers. The percentages excreted in the urine and feces were of the same magnitude for all volunteers (see Table

TABLE 1

Recovery values of radioactivity, expressed as the percentage of applied radioactivity

Volunteer no.	External recovery (%)	Percutaneous absorption		Total recovery (%)
		Urine (%)	Feces (%)	
1	96.0	0.312	0.007	96.3
2	97.4	0.700	0.013	98.1
3	98.7	0.215	0.010	98.9
mean	97.4	0.409	0.010	97.8
S.D.	1.4	0.257	0.003	1.3

1). Excretion was found to be almost complete after 5.5 days (see Fig. 3). These two phenomena were also encountered in the previous study. Therefore, it seems likely that the previously obtained lower value for the external recovery for one of the volunteers was due to an unnoticed loss of azone during the application period.

In an attempt to increase the total amounts of azone absorbed through human skin, the application area and time were extended. These parameters, however, do not affect the flux of penetrant as concentration, thickness of the deposited drug film and occlusion can do. It was shown for nitroglycerin that increasing the concentration in or the thickness of a transdermal device resulted in a decreased efficiency of percutaneous absorption (Wester, 1985). In this study both the concentration and the thickness of the deposited drug film were reduced by applying a reduced quantity of pure azone on an extended area. A further increase of the percutaneous absorption was anticipated to be obtainable by covering the application area with a membrane impermeable to water, the so-called occlusion. In this way transepidermal water loss is prevented and endogenous water is trapped in the stratum corneum. It is known that many substances penetrate better through hydrated skin than through the dry tissue. However, due to hydration the stratum corneum becomes somewhat thicker, which may decrease penetration (Blank, 1985). The actual percutaneous absorption of azone due to occlusion then, is the result of both phenomena.

Table 2 gives an overview of the various parameters in our two studies and also lists the percentages percutaneously absorbed, amounts absorbed and the fluxes. In the present study the absolute amounts and the percentages of azone absorbed had increased, but the flux was significantly lower. Thus, one can conclude that for azone the overall effect of the changes in occlu-

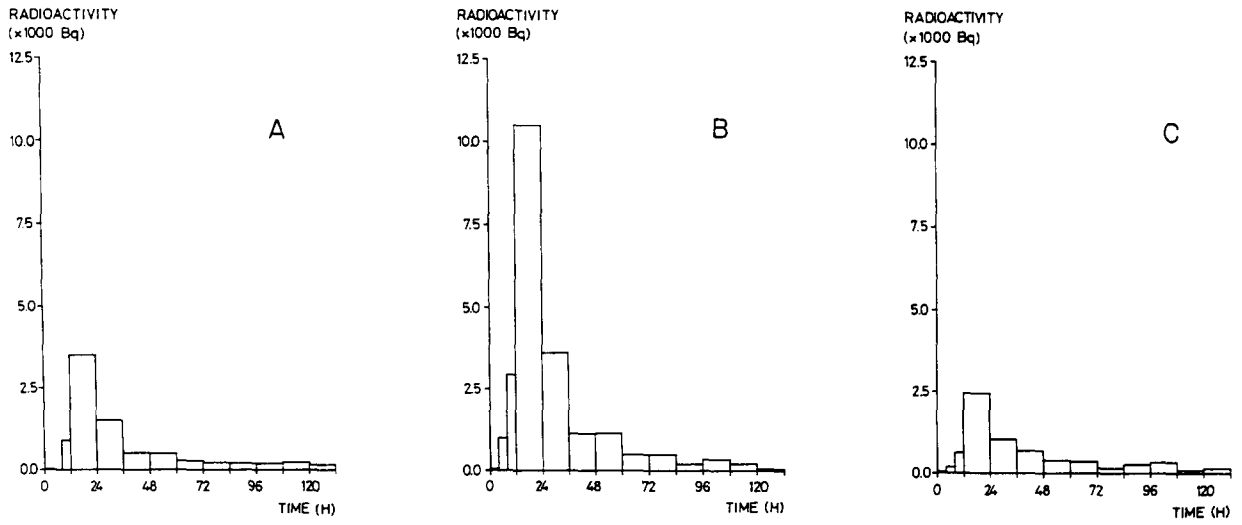

Fig. 3. Amounts of [^{14}C]azone-derived radioactivity per interval of time in urine. A, B, and C represent the data obtained for volunteer 1, 2 and 3, respectively.

TABLE 2

*Changes in study design compared to the former study * and its influence on the percutaneous absorption of azone through human skin*

Parameter	Unity	Previous study *	Present study
Dosage			
radioactivity applied	MBq	3.70	2.75
specific activity	GBq/mol	11.4	17.1
volume	µl	100	50
weight	mg	91.2	49.7 ± 5.1
concentration	mg/cm^2	5.7	2.1 ± 0.2
mean thickness	mm	0.063	0.023 ± 0.002
Application			
time	h	4	12
area	cm^2	16	24
occlusion		no	yes
Methods			
tape stripping		yes	no
Percutaneous absorption			
percentage	%	0.165 ± 0.055	0.491 ± 0.259
absolute amounts	µg	152.0 ±52.6	211.1 ±142.3
flux	µg/cm^2/h	2.38 ± 0.82	0.73 ± 0.49

Values are means ± S.D. Since the syringes used for the administration of the dosage were not weighed in the previous study, the S.D. of the weight, concentration and mean thickness could not be determined.

* See Wiechers et al., 1987.

sion, film thickness and concentration was negative.

Urinary excretion accounted for 97.2 ± 1.4% (S.D.) of the total excretion, which clearly shows that azone and/or its metabolites are mainly excreted by the kidneys. The urinary excretion profiles are depicted in Fig. 3. Notwithstanding varying amounts absorbed, excretion is almost complete within 3 days; between 87 and 94% of the total urinary excretion is recovered during the first 72 h. Thereafter, the amounts excreted are very low and virtually the same for all volunteers. These values are in accordance to previously obtained values.

By analysing tape stripping and urinary excretion data in the former study, it was assessed that the percutaneous absorption of azone was not the rate-limiting step in the clearance of azone, but that it was the formation or excretion of one or more metabolites. The absorption rate of azone then determines the slope of the initial rise of the urinary excretion rate plot, whereas the elimination of the metabolite(s) determines the slope of the terminal part of such a plot, in which the logarithm of the excretion rate is plotted against the midpoint time of the urine collection interval. In the present study half-lives were derived from such a plot to be 5.9 ± 3.9 (S.D.) and 22.3 ± 6.0 h for the rising and declining parts, respectively. These values closely correspond to previously obtained values (7.6 ± 3.4 and 23.8 ± 11.6 h, respectively). Therefore, the rate of percutaneous absorption was also not affected, despite the changes in study conditions.

The presence of metabolites in the urine which was indicated in the former study was confirmed by analysing the urine by HPLC, after concentrating the urine sample by lyophilization and extraction with methanol. This work-up procedure was necessary because of the very low amounts of radioactivity present in the large quantities of urine. If should be realized that this work-up bears the risk of losing radioactive material and/or individual components but, with a recovery of more than 80% in the final solution the procedure appeared to be satisfactory.

The metabolic profiles shown in Fig. 4 indicate the existence of at least 3 different polar components eluting within 20 min, a possible fourth one eluting at about 58 min, whereas unchanged azone,

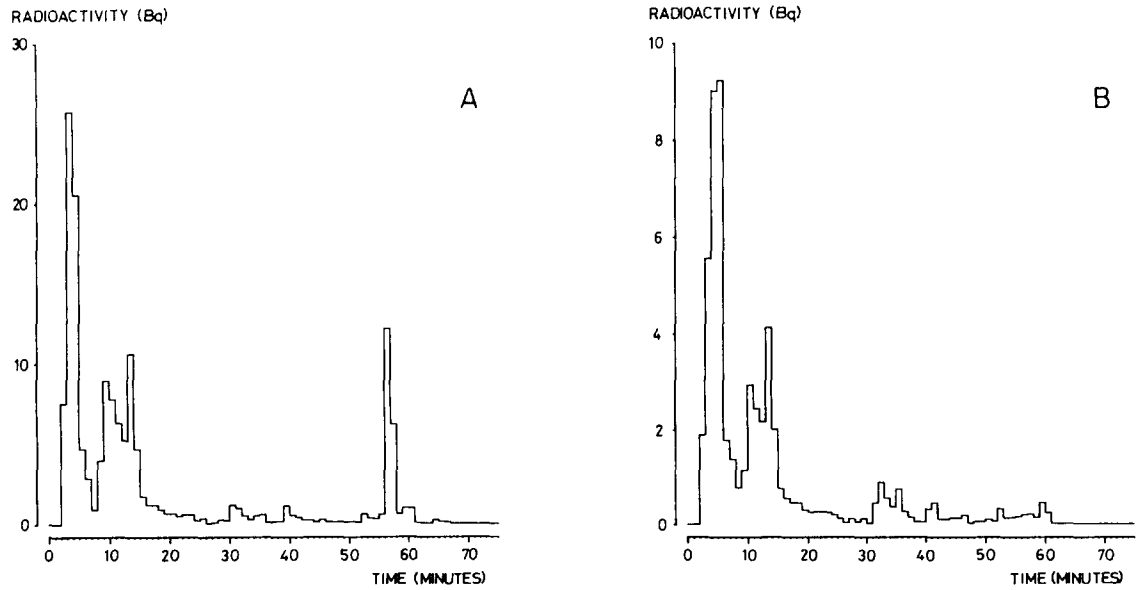

Fig. 4. Radiochromatograms of urine samples after dermal application of [^{14}C]azone under occlusion. A and B represent the metabolic profiles in the 12–24 h fraction of volunteers 1 and 3, respectively.

eluting at 63–64 min under these conditions is only present in minute amounts, if at all.

In both blood and plasma, concentrations of radioactivity were too low to allow accurate determination.

TABLE 3

Fecal excretion of [^{14}C]azone derived radioactivity (mean ± S.D., n = 3)

Volunteer no.	Sample no.	Time (h:min)	Radioactivity (Bq)
1	1	14:20	5.0±1.2
	2	33:19	18.8±3.3
	3	52:45	52.8±7.8
	4	73:54	62.2±2.8
	5	107:50	36.2±5.9
	Total		175.0
2	1	25:00	51.2±3.1
	2	59:45	125.0±9.6
	3	75:45	196.0±8.8
	4	105:30	53.1±5.4
	Total		425.3
3	1	21:40	16.8±1.2
	2	27:25	49.3±1.2
	3	52:25	96.1±4.0
	4	77:20	63.8±2.0
	5	101:30	85.1 (n = 1)
	Total		311.6

The amounts of radioactivity retrieved in the feces samples were very low as indicated in Tables 1 and 3. Excretion seemed to increase up to 75 h after application and to decline thereafter, but because of the limited number of data points no further conclusions could be drawn with respect to pharmacokinetic parameters.

No deviations in blood pressure, heart rate, respiration rate and body temperature were observed during the study period, nor were there any changes in clinical chemistry after completion of the experiment.

In conclusion, even after prolonged application to an extended area under occlusion conditions the percutaneous absorption of azone remained very low, without inducing pharmacological activity or irritation. Further studies with azone present in therapeutic drug formulations are in progress.

Acknowledgements

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