J. Soc. Cosmet. Chem., 40, 367–373 (November/December 1989)

Skin stripping as a potential method to determine *in vivo* cutaneous metabolism of topically applied drugs

JOHANN W. WIECHERS, RENELLA E. HERDER,
BEN F. H. DRENTH, and ROKUS A. de ZEEUW,
Groningen Centre for Drug Research, Bioanalysis and Toxicology Group,
University of Groningen, A. Deusinglaan 2,
9713 AW Groningen, The Netherlands.

Received August 25, 1989.

Synopsis

By chromatographing an extract of the tapes obtained in a skin stripping procedure, cutaneous metabolism of compounds after topical administration may be observable, provided that outward transdermal migration occurs. This method may be helpful, especially in situations where no differentiation between cutaneous and systemic metabolism can be made due to the experimental design or the very low systemic concentrations. Through use of this methodology, it can be assessed that the penetration enhancer for percutaneous absorption, Azone®, is only present as the parent compound in the stratum corneum, whereas the anti-acne agent Cyoctol undergoes cutaneous biotransformation during skin passage.

INTRODUCTION

In recent years there has been a renewed and growing interest in dermal and transdermal drug delivery. This route opens new possibilities for systemic therapy, especially for drugs with short biological half-lives due to extensive first-pass metabolism in the liver. Compounds, however, may also be metabolized in the skin before reaching the systemic circulation (1,2), thereby reducing their bioavailability.

For this reason, the cutaneous metabolism of these compounds should be studied and compared to already available systemic biotransformation data. If cutaneous metabolism occurs, additional investigations may be required to determine the pharmacological profile of the dermally formed metabolites.

A simple method to establish *in vivo* cutaneous metabolism of topically applied agents was developed and will be discussed on the basis of two compounds currently under investigation in our laboratories, Azone® and Cyoctol. Both compounds are to exert their action in human skin, Azone as a penetration enhancer for percutaneous absorp-

Johann W. Wiechers' present address is Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ, United Kingdom.

tion (3) and Cyoctol, an anti-androgen (4), as an anti-acne drug. In order to be able to follow the metabolic processes, tracer amounts of ^{14}C-labeled compounds were used. The structure of the compounds and the position of the labels is given in Figure 1.

MATERIALS AND METHODS

MATERIALS

^{14}C-labeled Azone, 1-dodecylazacycloheptan-2-one ([1-^{14}C]-dodecyl), and Cyoctol, 6-(5-methoxyphept-1-yl)bicyclo[3.3.0]octan-3-one ([^{14}C]-carbonyl), were kindly supplied by Nelson Research, Irvine, California, and Chantal Pharmaceutical Corporation, Los Angeles, California, respectively. The radiochemical purity was determined by isocratic high-performance liquid chromatography (HPLC) to be at least 95.3 and 97.0%, respectively, using the system described below. All other materials were HPLC grade and obtained commercially.

METHODS

Study performance. In separate studies Azone and Cyoctol were applied to a 24-cm^2 area on the volar aspect of the forearm of healthy human volunteers and left in place under occlusion for 12 and 8 hours, respectively. Azone was dosed in a therapeutic formulation (100 mg) to three volunteers at a concentration of 1.6%, containing tritium-la-

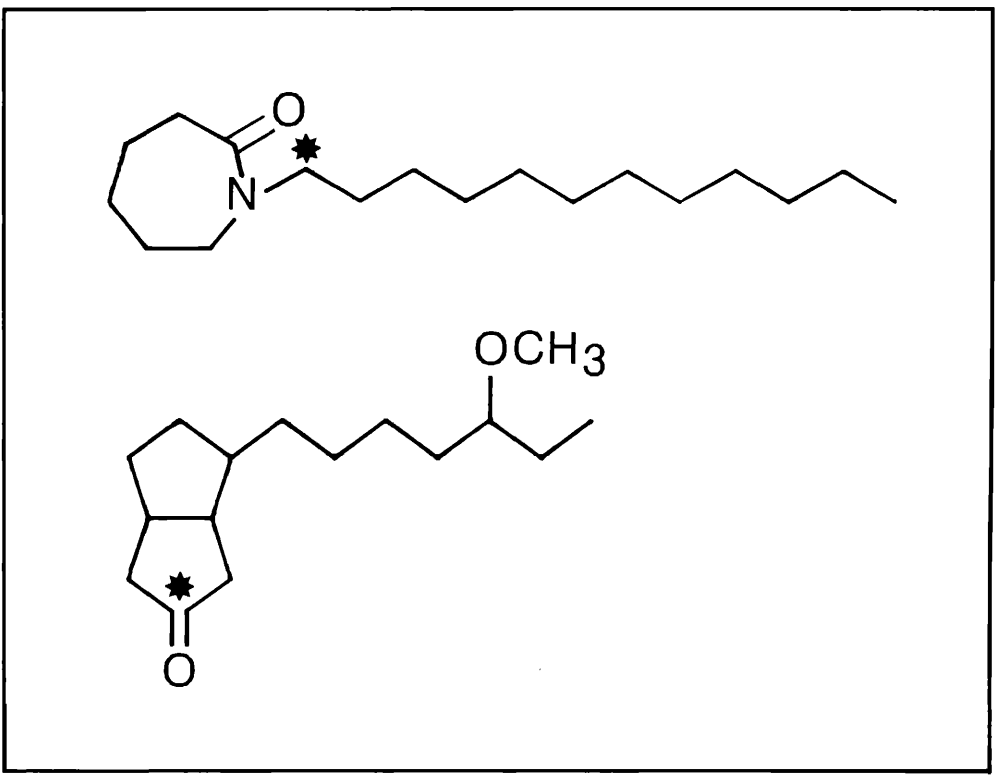

Figure 1. Structures of 1-dodecylazacycloheptan-2-one (Azone®, top) and 6-(5-methoxyhept-1-yl)bicyclo[3.3.0]octan-3-one (Cyoctol, bottom). The asterisks denote the position of the label.

beled triamcinolone acetonide as well, at a concentration of 0.05%. Cyoctol was dosed to four volunteers in an aqueous alcohol solution (ethanol (96%)/bidistilled water 75/25) at a concentration of 1.5%. Study conditions have been reported in full detail elsewhere (5,6). Skin samples were obtained at 1, 20, and 44 hours (Azone) or 1, 23, and 45 hours (Cyoctol) after removal of the dose by the skin stripping method described below.

Skin stripping. Skin stripping was done by means of commercial translucent cellophane tape of 9-mm width, made by 3M Company (Leiden, The Netherlands). Strips of ca. 6 cm in length were affixed and removed sequentially from the same transverse portion of the treatment site. At each stripping, the tape was firmly rubbed in place to achieve thorough adherence and then removed after about three seconds. The stripping procedure was complete when the area started to become glistening and the tape no longer adhered to the skin when applied, or when it became painful to the volunteer. Maximally 28 strips were applied.

All strips from one procedure were combined in a glass container, and 60 ml of methanol was added. The container was vigorously shaken for 16 hours to allow full extraction of drug-related material. The chemical stability in methanol of both compounds is at least several years. As it is impossible to spike tapes, the efficiency of extraction of radioactive material from the tapes cannot be given. At the end of the extraction period, however, the sticky layer of the tape had completely dissolved in the scintillation cocktail.

The extracts were evaporated to dryness under vacuum, and the residue was redissolved in methanol/phosphate buffer 0.01 M, pH 6.8 (85/15 v/v) (Azone), or in methanol (Cyoctol). After redissolution, the samples were filtered through a 0.45-μm filter. A 50-μl aliquot of the filtrate was injected into the HPLC system described below. The efficacy of the analytical procedure was checked. When a methanol solution containing tapes was spiked with ^{14}C-Azone or ^{14}C-Cyoctol and assayed identically, the recovery proved to be 94.7 ± 1.2 and 96.3 ± 2.4 (mean ± S.D.), respectively.

Metabolic profiling. Extracts of the dosages and the tapes were analyzed in 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, State College, Pennsylvania). Isocratic elution with methanol/phosphate buffer 0.01 M, pH 6.8 (85/15 v/v) was performed when analyzing the extracts of the dosage and the tapes containing ^{14}C-Azone-derived radioactivity. In the case of ^{14}C-Cyoctol-derived radioactivity, a linear gradient from 100% phosphate buffer 0.01 M, pH 6.8, to 100% methanol in 20 minutes followed by a methanol flush of 10 minutes was performed. In both cases the flow rate was 1.0 ml/min. Effluent fractions of 0.5 minutes were collected in polyethylene scintillation vials and vigorously shaken with 3 ml of the scintillation cocktail RiaLuma (Lumac, Landgraaf, The Netherlands). The samples were counted on a Packard Minaxi B4450 Liquid Scintillation Spectrometer (Packard Technologies, Irvine, California) for five minutes or a statistical accuracy of 0.5%.

RESULTS AND DISCUSSION

The chromatograms of the radioactivity in the dosages and the tape extracts are shown in Figures 2 and 3 for Azone and Cyoctol, respectively. The relative contribution of the peaks to the total eluted amount of radioactivity is given in Table I.

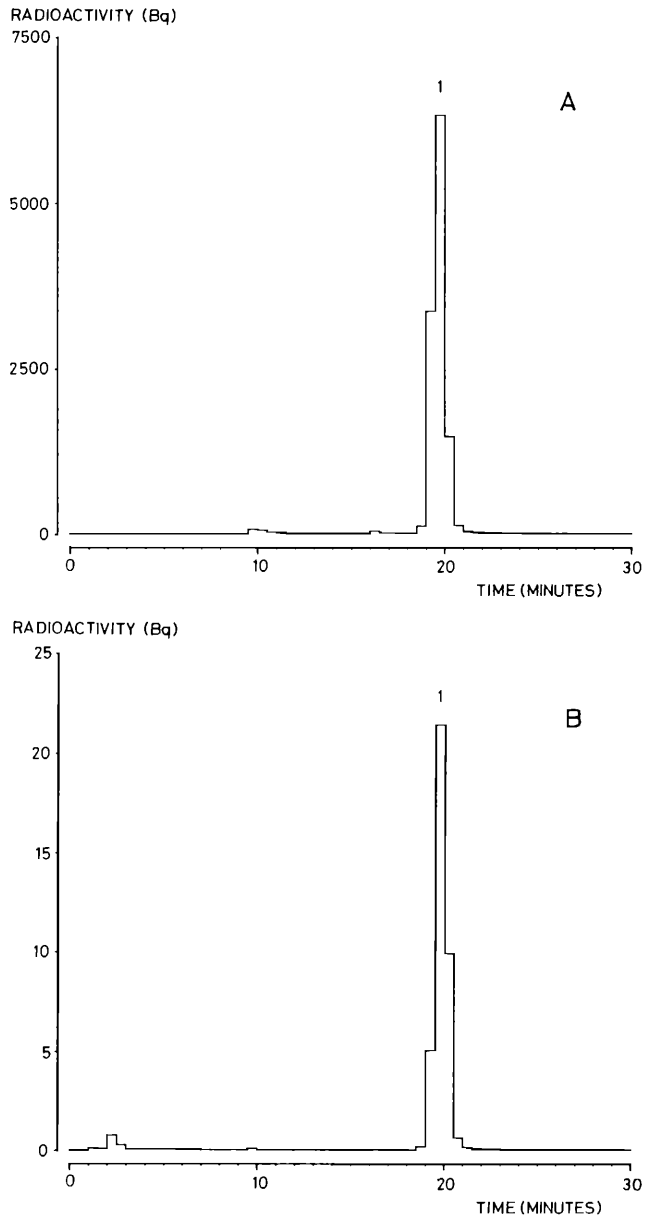

Figure 2. Radiochromatograms of ^{14}C-Azone-derived radioactivity in the dosage (A) and the tape extracts at 1 hour after removal of the dose (B).

The metabolic profile of the ^{14}C-Azone-derived radioactivity in the tape extract obtained from the stripping procedure at one hour after removal of the dose showed only the parent compound, except for a minor amount of radioactivity in the front. Tritiated triamcinolone acetonide was co-administered with the carbon-14–labeled Azone and was present in the tape extracts in large amounts relative to the amount of carbon-14 radioactivity (5). As the tritiated drug eluted at two minutes, the small peak at the

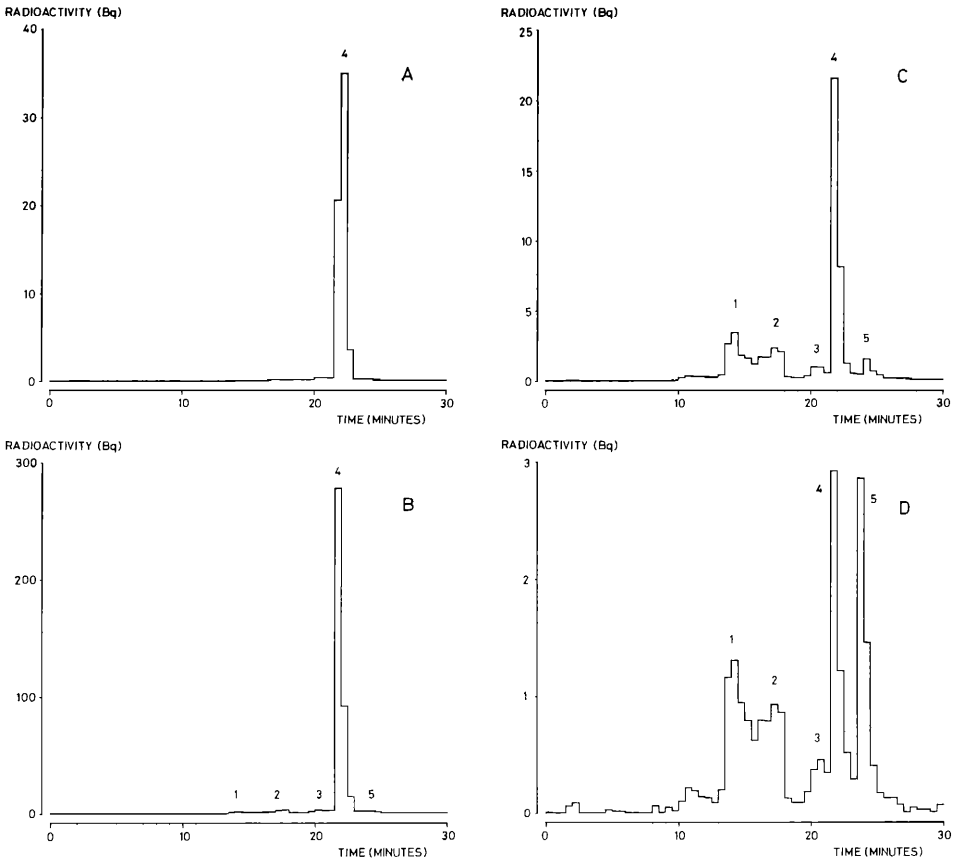

Figure 3. Radiochromatograms of ^{14}C-Cyoctol-derived radioactivity in the dosage (A) and the tape extracts at 1 (B), 23 (C), and 45 hours (D) after removal of the dose.

front most likely originated from the tritiated drug. It should be noted in this regard that even though liquid scintillation counters have programs to correct for spillovers when ^{14}C and ^{3}H isotopes are counted simultaneously, such programs may not be able to adequately correct if the amounts of ^{14}C isotopes present are rather small in comparison to the amounts of ^{3}H isotopes. Radiochromatograms at 20 and 44 hours showed similar profiles, yet at much lower quantities, due to the rapid disappearance of Azone from the stratum corneum (5). Levels of radioactivity were therefore close to the baseline, and this makes the assessment of the percentual contribution of individual peaks meaningless. Nevertheless, all profiles were basically the same at the three collection times, and it can therefore be concluded that only unchanged Azone is present in the stratum corneum.

With Cyoctol, the situation appears to be quite different. At one hour after removal of the dose, some metabolites can be detected in small amounts, but the majority of the radioactivity is still present as the parent compound (see Figures 3A and 3B). Table I shows that, as time goes by, the relative contribution of Cyoctol (peak 4), decreases, whereas that of the metabolites increases. At 45 hours after removal of the dose, only about 35% of the radioactivity in the stratum corneum is present as unchanged Cyoctol.

Table I

Relative Contribution of Individual Compounds as a Percentage of the Total Eluted Amount of ^{14}C-Azone (A; n = 3) or ^{14}C-Cyoctol (C; n = 4) Derived Radioactivity (mean ± SD)

Test compound	Peak number[1]	Dosage	Relative contribution (%) in tape extracts at hour		
			1 (A + C)	20 (A) or 23 (C)	44 (A) or 45 (C)
Azone	1	95.4 ± 1.8	94.0 ± 3.8	N.D.[2]	N.D.[2]
Cyoctol	1		1.4 ± 0.9	9.7 ± 5.0	17.5 ± 3.9
	2		4.0 ± 2.6	12.2 ± 4.2	15.7 ± 1.7
	3		4.0 ± 3.1	8.3 ± 7.0	5.9 ± 1.6
	4	93.9 ± 1.4	78.3 ± 16.8	49.5 ± 19.7	35.4 ± 13.4
	5		6.6 ± 7.6	9.2 ± 5.8	14.2 ± 10.0

[1] The numbers correspond to those in Figures 2 and 3.
[2] N.D. = not determined.

The possibility of chemical degradation of Cyoctol during the sample work-up could be excluded by having the parent compound undergoing the same sample processing. No compounds other than Cyoctol could be detected.

These findings indicate that only unchanged Azone is present in the tape extracts, while in the case of Cyoctol, both the parent compound and its metabolites can be found. The stratum corneum, however, is a layer of dead cells, assumed to be devoid of metabolic activity, as opposed to the underlying viable epidermis and dermis where skin metabolism may take place (1). Bioconversion of Cyoctol, therefore, conceivably occurred in one of these layers. Yet, stripping removes only two thirds of the stratum corneum (7) and cannot have removed part of the viable epidermis. The presence of metabolites in the stratum corneum can be explained by assuming outward migration of the metabolites formed in the viable epidermis and/or dermis. Outward migration has been described for compounds following oral administration (8,9), but recently could be established following dermal application as well in the case of Cyoctol (6,10). Although the majority of the metabolites formed in the viable epidermis will move inwards into the body, favored by a more aqueous environment and systemic removal, a concentration gradient will also exist towards the stratum corneum. As a result of that, the stripping technique will usually underestimate the extent of metabolism. In the case of Cyoctol, for instance, De Zeeuw *et al.* showed that this drug was completely metabolized during skin passage to a more nonpolar metabolite, corresponding to peak 5 in Figure 1 (10).

A good indication as to cutaneous metabolism can be obtained from the metabolic profiles of the ipsi- and contralateral plasma samples (11). However, it is sometimes impossible to apply the latter methodology, for example, when dosing on areas such as the back, abdomen, or forehead. Moreover, the levels of radioactivity in the ipsi- and contralateral plasma samples have to be relatively high to obtain reliable metabolic profiles. This presents severe difficulties with drugs that have low dermal absorption such as Azone (5,12,13).

The skin stripping methodology does not have these disadvantages and thus seems to be an interesting, simple, and noninvasive alternative to assess *in vivo* cutaneous metabolism, provided that sufficient outward migration of metabolites occurs. The technique

should be considered as a potential method to determine whether metabolism can take place rather than providing quantitative information on the extent of cutaneous metabolism.

ACKNOWLEDGMENTS

This study was supported in part by Nelson Research and Chantal Pharmaceutical Corporation.

REFERENCES

(1) R. H. Guy, J. Hadgraft, and D. A. W. Bucks, Transdermal drug delivery and cutaneous metabolism, *Xenobiotica,* 17, 325–343 (1987).

(2) R. J. Martin, S. P. Denyer, and J. Hadgraft, Skin metabolism of topically applied compounds, *Int. J. Pharm.,* 39, 23–32 (1987).

(3) R. B. Stoughton and W. O. McClure, Azone®: A new non-toxic enhancer of cutaneous penetration, *Drug Devel. Ind. Pharm.,* 9, 725–744 (1983).

(4) L. C. Ford, H. A. Hammill, R. J. DeLange, D. A. Bruckner, F. Suzuki-Chavez, K. L. Mickus, and T. B. Lebherz, Determination of estrogen and androgen receptors in *Trichomonas vaginalis* and the effects of antihormones, *Am. J. Obstet. Gynecol.,* 156, 1119–1121 (1987).

(5) J. W. Wiechers, B. F. H. Drenth, J. H. G. Jonkman, and R. A. de Zeeuw, Percutaneous absorption, metabolic profiling and excretion of the penetration enhancer Azone® after multiple dosing of an Azone-containing triamcinolone acetonide cream (TAZ) in humans, *J. Pharm. Sci.,* 79, 111–115 (1990).

(6) J. W. Wiechers, R. E. Herder, B. F. H. Drenth, and R. A. de Zeeuw, Percutaneous absorption, disposition, and excretion of ^{14}C-labeled Cyoctol in humans after a single dermal application, *Int. J. Pharm.* (in press).

(7) M. T. Hojyo-Tomoka and A. M. Kligman, Does cellophane tape stripping remove the horny layer? *Arch. Dermatol.,* 106, 767–768 (1972).

(8) C. C. Peck, D. P. Conner, B. J. Bolden, R. G. Almirez, T. E. Kingsley, L. D. Mell, M. G. Murphy, V. E. Hill, L. M. Rowland, D. Ezra, T. E. Kwiatkowski, C. R. Bradley, and M. Abdel-Rahim, Outward transcutaneous chemical migration: Implications for diagnostics and dosimetry, *Skin Pharmacol.,* 1, 14–23 (1988).

(9) C. C. Peck, D. P. Conner, B. J. Bolden, R. G. Almirez, L. M. Rowland, T. E. Kwiatkowski, B. A. McKelvin, and C. R. Bradley, "Outward Transdermal Migration of Theophylline," in *Pharmacology and the Skin,* B. Shroot and H. Schaefer, Eds. (Karger, Basel, 1987), Vol. 1, pp. 201–208.

(10) R. A. de Zeeuw, R. E. Herder, J. W. Wiechers, and B. F. H. Drenth, Metabolic conversion of Cyoctol during skin passage in humans, *Pharm. Res.* (in press).

(11) A. Yacobi, R. A. Baughman, D. B. Cosulich, and G. Nicolau, Method for determination of first-pass metabolism in human skin, *J. Pharm. Sci.,* 73, 1499–1500 (1984).

(12) J. W. Wiechers, B. F. H. Drenth, J. H. G. Jonkman, and R. A. de Zeeuw, Percutaneous absorption and elimination of the penetration enhancer Azone in humans, *Pharm. Res.,* 4, 519–523 (1987).

(13) J. W. Wiechers, B. F. H. Drenth, J. H. G. Jonkman, and R. A. de Zeeuw, Percutaneous absorption, metabolism, and elimination of the penetration enhancer Azone in humans after prolonged application under occlusion, *Int. J. Pharm.,* 47, 43–49 (1988).