Pharmaceutical Research, Vol. 7, No. 6, 1990

Report

Metabolic Conversion of Cyoctol During Skin Passage in Humans

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Received July 19, 1989; accepted December 15, 1989

Upon application of ^{14}C-labeled cyoctol to the forearm of healthy volunteers, no parent cyoctol was detectable in ipsilateral blood plasma. The ^{14}C activity was largely accounted for by a component with higher lipophilicity than the parent compound, as justified from their HPLC retention. Thus, this study suggests that human skin is capable of nearly complete cutaneous first-pass metabolism, resulting in negligible systemic availability of cyoctol. In a comparable experiment, rabbits were also able to convert cyoctol during skin absorption to a more lipophilic metabolite, which was identified as the palmitoleic acid ester of *O*-demethylated cyoctol by GC/MS. However, chromatographic evidence indicates that the human ipsilateral metabolite differs from the rabbit cyoctol metabolite.

KEY WORDS: cutaneous metabolism; cutaneous first pass; humans; cyoctol; palmitoleic ester metabolite.

INTRODUCTION

The skin can serve as a route for delivering drugs for local or systemic effects. Although it is recognized that drugs may undergo metabolism during skin passage, the metabolic capacity of the skin is thought to be rather limited, and transdermal drug delivery may, to a large extent, avoid the first pass effect of the gastrointestinal tract and the liver (1–7).

Cyoctol, or 6-(5-methoxyhept-1-yl)bicyclo[3.3.0]octan-3-one) (Chantal Pharmaceutical Corporation, Los Angeles, CA), is a topical androgen receptor blocking agent, currently undergoing clinical investigations for the treatment of acne vulgaris, alopecia, and keloid scar tissue. This paper describes the complete metabolic conversion of cyoctol during skin passage after topical application to the forearm of human volunteers.

MATERIALS AND METHODS

Chemicals

^{14}C-Labeled cyoctol was obtained from Chantal Pharmaceutical Corporation. The chemical structure and the position of the label is indicated in Fig. 1. The radiochemical and chemical purity of the test compound was tested by RP-HPLC and found to be better than 97.0%.

The stock solution of ^{14}C-cyoctol in ethanol (96%)–distilled water, 75/25 (v/v), contained approximately 1.5% cyoctol. The specific activity was 12.1 mCi/mmol.

All other chemicals were of analytical grade and obtained from Merck, Darmstadt, FRG, unless specified otherwise.

Study Design

The study was set up to investigate the absorption, disposition, metabolism, and excretion of cyoctol in humans after a single dermal application. Four young healthy male volunteers participated. Prior to the study, each volunteer was subjected to a standard physical examination and a medical history was taken. All volunteers gave their written informed consent. The studies were performed in accordance with FDA's GCP and GLP regulations and guidelines.

The study was done with a single application of approximately 60 μl of the stock solution, containing approximately 35 μCi of labeled cyoctol. The application area on the volar aspect of the right forearm was shaved 4 days prior to the start of the study. The application area was outlined by an adhesive template, 10 × 14 cm (Ensure-it, Deseret Medical Inc., Sandy, UT), from which a rectangle of 4 × 6 cm had been removed in the center. Dosing was done using a glass microsyringe of 100 μl and the solution spread evenly over the 4 × 6-cm area with a metal spatula. Thereafter, the area was protected by a nonocclusive dome and the dosage left in place for 8 hr.

After this time period, during which the volunteers were seated with their arms resting on a bench, the dome and template were taken off and the remainder of the dose was removed by wiping the skin with gauze pads, then by rinsing with ethanol sponging six times. The treated area was left uncovered for 1 hr, after which one-third was tape stripped and covered with a dry gauze pad until the next tape strip-

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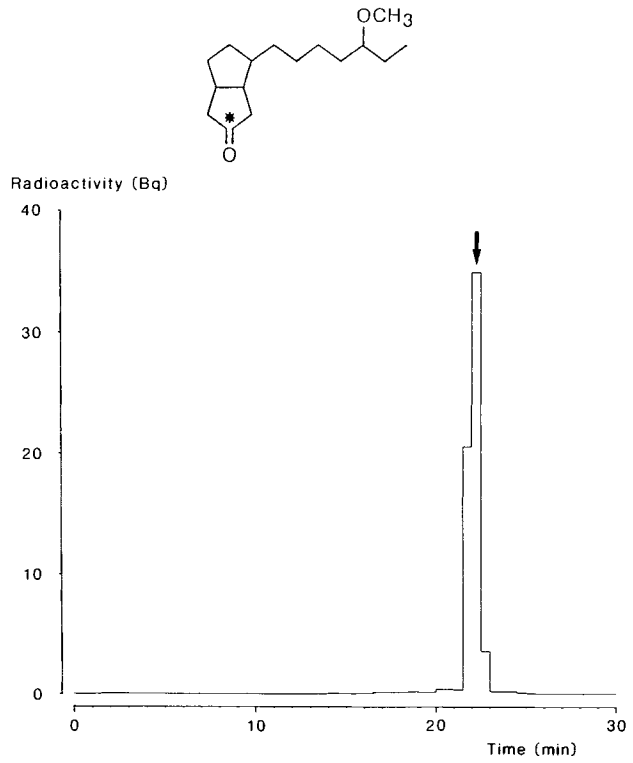


Fig. 1. Structure of cyoctol and elution profile in the HPLC analysis using a linear gradient (A) from 100% phosphate buffer, 0.01 *M*, pH 6.8, to 100% methanol in 20 min and a 10-min methanol flush. The asterisk marks the position of the ^{14}C label.

ping (see below). All items expected to contain radioactivity were saved for analysis.

Sample Collection and Treatment

All urine and feces samples were collected up to 120 hr after removal of the dose. Also, at regular intervals, blood samples were drawn from the cubital veins in both arms simultaneously, i.e., ipsilaterally (the treated, right arm) and contralaterally (left arm) (8). Blood samples of 10 ml were collected in heparinized glass tubes and centrifuged to obtain the plasma. All samples were stored at −20°C until analysis.

Three tape strippings, each on about one-third of the treated area, were done at 1, 23, and 45 hr, respectively, after removal of the dose. This was done by commercial cellophane adhesive tape of 9-mm width (3M Company, Leiden, The Netherlands). In order to remove most of the stratum corneum, 28 strips, approximately 6 cm in length, were sequentially adhered to and removed from the same transverse portion of the treatment site. Each individually numbered strip was firmly rubbed in place to achieve thorough adherence and removed in a slow and even fashion after about 3 seconds. This procedure was repeated on days 2 and 3 on the adjacent one-third areas, so that at the end of the third day virtually 100% of the application area had been stripped of its stratum corneum (9).

Urine samples were filtered through a paper filter and then through a 0.45-μm membrane filter (Millipore, Etten-Leur, The Netherlands) to remove particulate matter. Aliquots of 2 ml were subjected to HPLC analysis.

Feces were freeze-dried and homogenized and the resulting powder was combusted to determine radioactivity. As fecal excretion of radioactivity was negligible no further analyses were done.

Preliminary total radioactivity analysis of plasma samples indicated that ipsilateral levels were rather low (maximum values were observed after 6–10 hr and did not exceed 8 Bq/ml) and that contralateral levels were only 1/8–1/10 of the corresponding ipsilateral values. As a result, the large majority of the contralateral samples was around the background level of blank plasma (0.3 Bq/ml) (10). Therefore, in order to have sufficient radioactivity for metabolic profiling only ipsilateral samples were taken that contained more than 1 Bq/ml. This was the case for the majority of the samples taken at 2, 4, 6, 8, 10, and 12 hr, i.e., during and shortly after the exposure. These samples were pooled from all volunteers. To the total volume of 60 ml, the same amount of methanol was added and, after thorough mixing for 5 min, centrifugation was carried out at 75,000*g* for 30 min (Ultracentrifuge L8-55, Beckman, Irvine, CA). The supernatant was carefully removed and evaporated at 30°C under reduced pressure to a volume of about 1 ml. After centrifugation for 5 min at 15,000*g* (Biofuge A, Heraeus Christ, De Bilt, The Netherlands), the resulting supernatant was filtered through a 0.45-μm membrane filter (Millipore). Aliquots were subjected to HPLC analysis.

Tape-stripping samples were pooled per volunteer per day and extracted with 60 ml methanol. The extracts were evaporated to dryness at 30°C under reduced pressure, the residues redissolved in 1–2 ml methanol, and the solutions filtered through a 0.45-μm membrane filter. Aliquots were subjected to HPLC analysis.

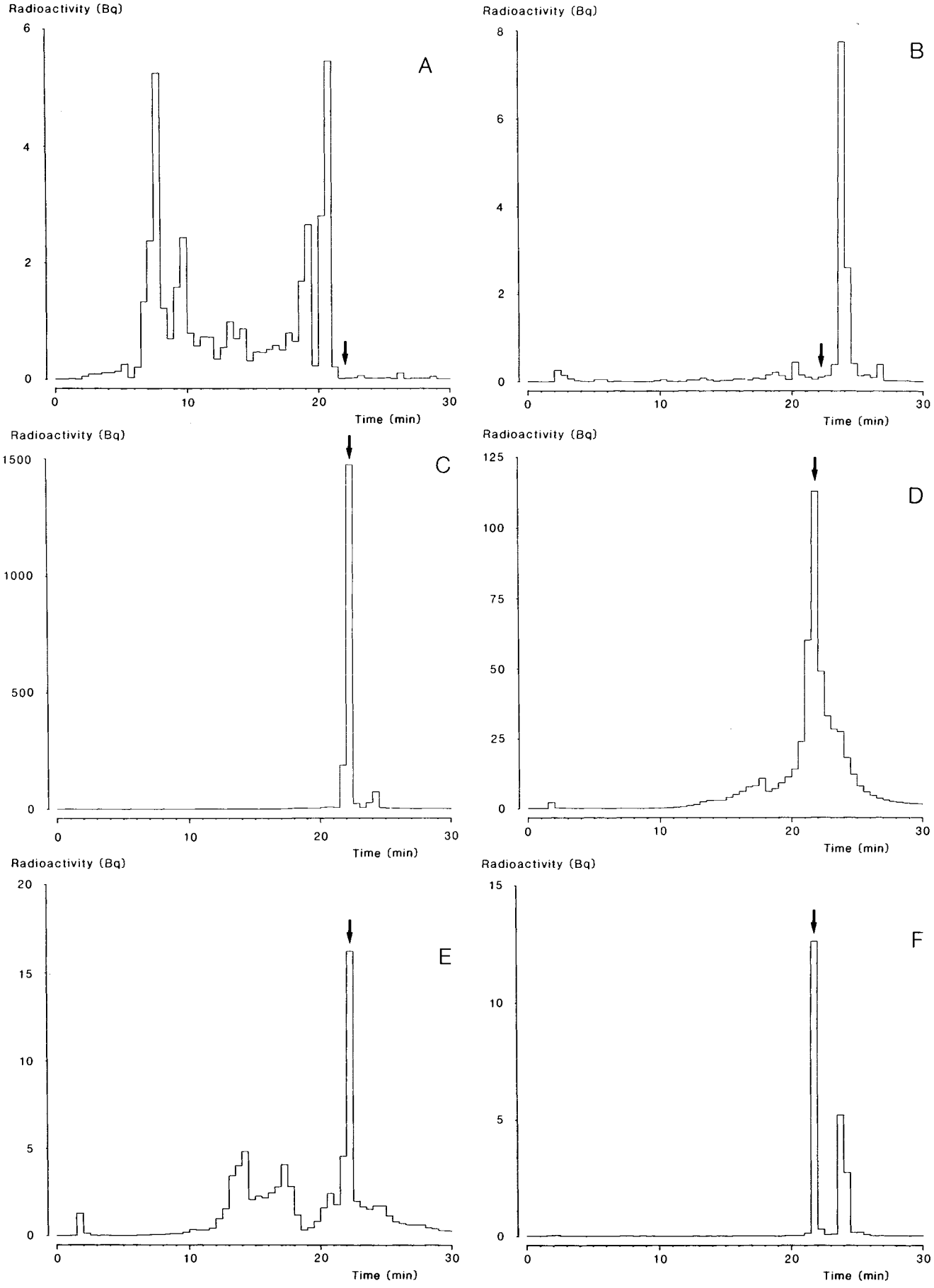
The gauze pads used for the external recovery of nonabsorbed cyoctol were each extracted with 60 ml methanol. The methanol extracts were filtered through a 0.45-μm membrane filter and aliquots were subjected to HPLC analysis.

HPLC Analysis

For the analysis of ^{14}C-cyoctol and its metabolites a system was used consisting of two Waters M510 pumps, a stainless-steel column (150 × 4.6 mm, i.d.), packed with Nucleosil C_{18}, 5-μm particle size (Macherey-Nagel, Düren, FRG), and a Rheodyne injection valve, Model 7125 (Inacom, Veenendaal, The Netherlands), capable to accept loops of different volumes. The system was controlled by an Adalab data acquisition/control device (Interactive Microware, State College, PA).

A linear gradient (A) from 100% phosphate buffer, 0.01 *M*, pH 6.8, to 100% methanol in 20 min, followed by a methanol flush of 10 min was capable of eluting components of vastly different polarities within an acceptable time. Alternatively, a shallow linear gradient (B) starting at 60% methanol in phosphate buffer to 100% methanol in 60 min was used to study the more lipophilic components. Flow rates were always 1.0 ml per min.

Column effluent fractions of 0.5 ml were collected by a Helirac fraction collector (LKB, Zoetermeer, The Netherlands). Each fraction was mixed with 3 ml RiaLuma (Lumac, Landgraaf, The Netherlands) and counted in a Minaxi B4450 liquid scintillation counter (Packard Instruments, Gronin-


Radioactivity (Bq)
A
Time (min)
Radioactivity (Bq)
B
Time (min)
Radioactivity (Bq)
C
Time (min)
Radioactivity (Bq)
D
Time (min)
Radioactivity (Bq)
E
Time (min)
Radioactivity (Bq)
F
Time (min)

gen, The Netherlands) for 5 min or a statistical accuracy of 0.5%, whichever came first. Data were converted to disintegrations per second (Bq) after assessing the extent of quenching by using an external standard.

RESULTS AND DISCUSSION

The total recovery of radioactivity (mass balance) was essentially 100%. Percutaneous absorption of cyoctol as assessed by the amounts of radioactivity in the urine and feces was around 6% of the applied dose. The remaining 94% was retrieved from the application site after the 8-hr exposure. Urinary excretion was by far predominant. More details on these aspects will be reported separately (10).

All biomaterials tested were stable during this study. They were stored at −20°C and thawed/refrozen several times. Also, cyoctol added to heparinized blood and plasma was found stable under these conditions. Using gradient A, the parent ^{14}C-cyoctol eluted between 21 and 23 min, as shown in Fig. 1. Day-to-day variations in rentention times were in the order of 0.5 min. Analysis of the urine samples indicated that virtually no cyoctol was excreted as such and that the compound was converted to a series of more polar metabolites, eluting between 6 and 21 min. A typical urinary metabolic profile is shown in Fig. 2A, the arrow indicating the position of unchanged cyoctol. The small amount of radioactivity eluting between 21 and 21.5 min represents the tail of the major peak eluting between 20 and 21 min and not parent cyoctol, as could be shown by subjecting this sample to gradient (B).

Analysis of the pooled, ipsilateral plasma samples with the same gradient resulted in the metabolic profile depicted in Fig. 2B. The parent compound was undetected, and most radioactivity was present in the fractions eluting between 23 and 25 min. As the ipsilateral site is right behind the application area, the parent compound must have been completely metabolized during the percutaneous absorption, either in the skin or on the skin surface, before systemic metabolism could have occurred. The impact of the systemic metabolism is reflected in the urine profile in Fig. 2A.

If degradation or bacterial attack had taken place on the skin, it would have been noticeable in the external recovery of the dose removed from the skin after exposure. Figure 2C, depicting the external recovery of volunteer 4, removed by ethanol sponging, indicates that this is not the case as the approximately 95% of the radioactivity is still present in the parent peak. Nevertheless, a small peak can be seen between 23.5 and 24.5 min. It should be noted that the latter peak was also present in the tape strippings, together with the parent compound and metabolites of a more polar nature, eluting between 10 and 20 min. Moreover, the metabolic peaks became more abundant as relative to parent cyoctol in the tape strippings from day 1 to day 2 to day 3. Figure 2D depicts the metabolic profile in the tape strippings on day 1, 1 hr after removal of the dose. The differences with Fig. 2C, i.e., the dose remaining on the skin 1 hr earlier, are quite evident. Figure 2E represents the metabolic profile of the tape strippings at day 3. The parent compound now comprises only 28% of the total radioactivity eluted.

While the stratum corneum consists of dead keratinized cells, metabolizing activity in the stratum corneum can still occur (11). This may explain the occurrence of cyoctol metabolites in the external recovery and the tape strippings. However, it is also conceivable that these metabolites are due to back diffusion from the viable epidermis and/or dermis. The latter has also been called outward transdermal migration (12). This appears to be corroborated by the fact that the relative abundance of the metabolites increases with time.

Unfortunately, the very low amounts of material present in the ipsilateral plasma samples did not allow identification of the lipophilic metabolite because coeluting nonradioactive material prevented adequate mass spectra. However, in an attempt to obtain more information on the possible metabolic pathways leading to more lipophilic compounds and their identification, a high dose of ^{14}C-cyoctol was applied topically to the ear of rabbits and left in place for 6 hr, and ipsilateral plasma samples were drawn from the ear vein at that time (13). Metabolic profiling of the latter samples by gradient A indeed showed the presence of a more lipophilic metabolite, eluting with the same retention time as observed in the human samples, namely, 23–25 min (see Fig. 2F). It could also be seen that in the rabbit the cutaneous first-pass effect was not 100%, but this may have been due to the much higher dose given to the animals, which was far beyond the therapeutic range. Analysis by gas chromatography–mass spectrometry revealed the rabbit metabolite to be the palmitoleic acid ester of *O*-demethylated cyoctol, with a molecular weight of 474. This structure suggests *O*-demethylation of the 5-methoxy group as a Phase I reaction, followed by palmitoleic acid conjugation as a Phase II reaction.

Since HPLC gradient A is not suitable to distinguish between substances with small differences in polarity, other elution systems were applied to compare the rabbit and human lipophilic metabolites.

As judged by their retentions with the shallow gradient of system B, the rabbit metabolite was more lipophilic (eluting at 35–38 min) than the human lipophilic metabolite seen in the external recovery (eluting at 25–28 min) (Fig. 3). A direct comparison with the human lipophilic metabolite in ipsilateral blood was not possible because of the lack of further human material.

These results demonstrate that cyoctol was completely

Fig. 2. Metabolic profile of human and rabbit samples. (A) Urine from volunteer 1, collected between 4 and 8 hr. (B) Pooled ipsilateral human plasma. (C) External recovery (dose removed from the skin after an exposure of 8 hr) from volunteer 4. (D) Tape strippings on day 1 (1 hr after removal of the dose) from volunteer 2. (E) Tape strippings on day 3 (45 hr after removal of the dose) from volunteer 2. (F) Ipsilateral rabbit plasma. All profiles were obtained with gradient A (see text and Fig. 1). Arrows indicate the position of unchanged cyoctol. The latter ran off-scale in Fig. 2E.

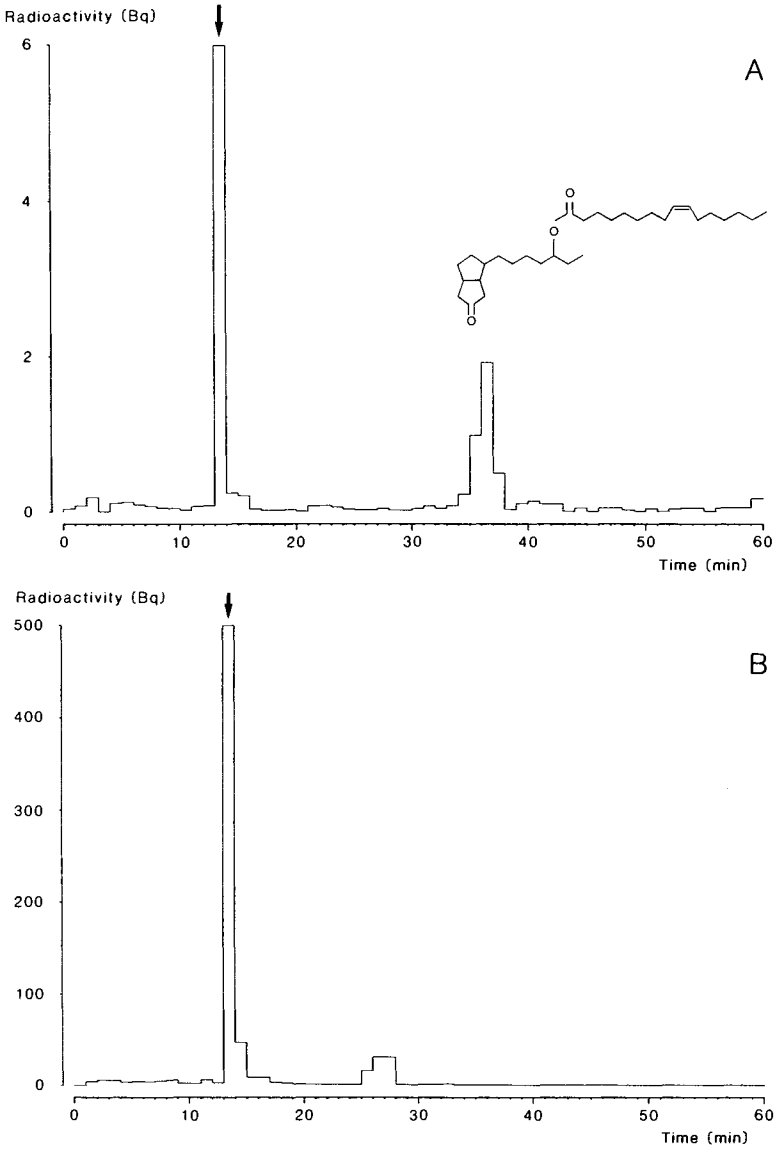


Fig. 3. Metabolic profiles using a shallow linear gradient (B), starting elution at 60% methanol in phosphate buffer, 0.01 *M*, pH 6.8, to 100% methanol in 60 min. (A) Ipsilateral rabbit plasma. (B) Cyoctol and the lipophilic metabolite isolated from external recovery of volunteer 4. Arrows indicate the position of unchanged cyoctol. The lipophilic rabbit metabolite was identified as indicated.

metabolized during skin passage. Although it is recognized that the skin has the potential to metabolize drugs, notably the viable epidermis, its capacity to do so is considered to be rather low (3,4,6), particularly in humans (7). While a major benefit of transdermal drug delivery is the circumvention of gastrointestinal and/or hepatic first-pass metabolism, our present findings show a large cutaneous first-pass effect for cyoctol. It is remarkable that the resulting metabolites are more lipophilic than the parent compound. A more extensive evaluation of the metabolizing capacity of the skin *in vivo* is necessary, especially in humans.

On the other hand, the above findings may support the use of cyoctol as a topical drug since it should exert its activity in the skin without giving systemic antiandrogen side effects. The latter may indeed be the case if the ipsilateral metabolite can be shown to be devoid of such activity.

ACKNOWLEDGMENT

This work was supported by Chantal Pharmaceutical Corporation.

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