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Diphencyprone Is Not Detectable in Serum or Urine Following Topical Application

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Diphencyprone is a potent contact sensitizer in widespread use for treatment of alopecia areata. It is currently not known whether this compound is absorbed following topical application. This is important, since little is known regarding potential toxicity.

We therefore analysed serum and urine samples following application of at least 0,5 ml of a 1% solution of diphencyprone to the scalp of patients under treatment for alopecia areata. Serum samples were obtained over 8 h following treatment and 24-h urine collections were performed.

The threshold for detection was 2 ng, and the assay gave an accurate linear response for samples of serum and urine containing known concentrations of diphencyprone. Blood and/or urine samples were obtained from a total of 18 subjects. Diphencyprone was not detected in any sample of serum or urine from the subjects. These data suggest that diphencyprone is not absorbed following application to the skin. ***Key words: diphenylcyclopropenone; alopecia areata; toxicity.***

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The induction of contact allergic dermatitis is an effective, widely used treatment modality for alopecia areata. Three sensitizers have principally been employed: dinitrochlorobenzene (DNCB) (1, 2), squaric acid dibutylester (SADE) (3, 4) and diphencyprone (diphenylcyclopropenone, DPCP) (5, 6), see Fig. 1. DNCB has the disadvantage of mutagenicity, demonstrating a positive Ames test (7). Furthermore, in a study using radiolabelled DNCB it was estimated that 53% of the dose applied to the skin was absorbed and ultimately excreted in the urine (8). SADE has a short shelf-life rendering it impractical for routine use. DPCP is a reliable sensitizer (9) which is not mutagenic (10), though contaminants and short-lived photoproducts may have this potential, and has a convenient shelf-life. It has therefore become the most popular sensitizer. Although controlled trials have demonstrated the efficacy of this compound (5, 6), no studies of absorption or toxicity have been performed.

We therefore considered it important to determine whether DPCP could be detected in serum or urine following topical treatment.

METHOD

Subjects

Subjects were selected from among patients attending our department weekly for treatment of alopecia areata with DPCP. All subjects had been under treatment for at least 4 weeks. Only patients requiring treatment with a 1% DPCP solution to maintain a mild dermatitis were recruited. Since this is the highest concentration used in our department, these patients were considered to be at the greatest risk from absorption. Informed consent was obtained.

Treatment

A minimum of 0.5 ml of a solution containing 1% (w/v) DPCP in a mixture of industrial methylated spirit 95% and propylene glycol (9:1 by volume) was applied to the scalp.

Sampling

Initially, single 10-ml blood samples were obtained from a series of subjects 30–60 min after the solution had been applied. After these had been analysed, series of blood samples were obtained from subjects at each of the following time points following application: 15, 30, 60, 90 min, 2, 4 and 8 h. In addition, a series of 24-h urine specimens was obtained, each commencing immediately before treatment. All blood samples were taken in plain glass bottles; the serum was separated and frozen promptly at −20°C. Urine collections were kept refrigerated and returned to the hospital on the day following treatment. To minimise photodecomposition, samples were stored in the dark prior to analysis.

Assay

High-pressure liquid chromatography was employed to detect DPCP. A Hypersil ODS 5 Ug column (30×0.4 cm) was used. The eluent was 65% acetonitrile in distilled water; the flow rate was 1 ml/min; and the wavelength of the assay was 295 nm. Ambient temperature was used, and sensitivity 0.2 AUFS. The retention time was 4.5 min.

Extraction of DPCP from serum and urine

Samples of 200 µl serum and 400 µl acetonitrile were vortex-mixed for 30 s in an Eppendorf tube and then centrifuged at 12,000 rev/min for 4 min. A 100-µl aliquot of supernatant was injected into the chromatograph.

Samples of 200 µl of urine and 1 ml dichloromethane were vortex-mixed for 60 s, centrifuged at 3,000 rev/min for 5 min and the aqueous phase discarded. Eight hundred µl of the organic phase were transferred to a clean glass tube and evaporated to dryness under nitrogen at 50C. The residue was reconstituted in 100 µl acetonitrile and 50 µl were injected into the chromatograph. The extraction was then repeated scaled up to 1 ml of urine.

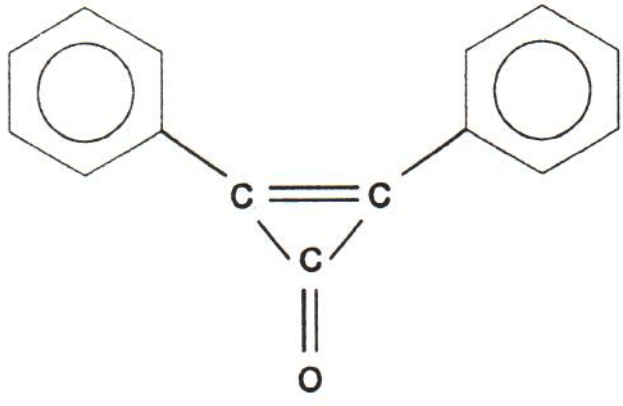

Fig. 1. Structure of diphencyprone.

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This extraction method was also used to increase the sensitivity of the serum assay for samples taken at 15 and 60 min post application in one subject.

RESULTS

Performance of the assay

For both serum and urine, evaluation of the assay using known concentrations of DPCP, showed a linear response up to 10 μg/ml passing through zero. In serum, coefficients of variation at concentrations 0.5, 1.0 and 10 μg/ml were 4.0, 1.0 and 2.5%, respectively ($n = 5$ for each concentration). In urine, coefficients of variation at 1.0 and 10 μg/ml were 4.1 and 1.9%, respectively ($n = 6$ at each concentration). Scaling up the assay to 1 ml urine produced no deterioration of performance. There was no interference from endogenous serum or urine constituents. The minimum detection level was 2.0 ng injected into the column.

Stability studies on serum indicated that, at concentrations of 0.5 and 10 μg/ml, 10% loss occurred at room temperature in 5 h and up to 30% after storage at −20°C for 10 days. Up to 40% DPCP was lost after storage for 4 weeks at −20°C. All samples were therefore analysed within 24 h of receipt by the laboratory.

Analysis of serum and urine samples

A total of 18 subjects (11 males, 7 females) participated in the study. Ages ranged from 16 to 54 years. Single blood samples were obtained from 7, complete blood profiles from 7, and 24-h urine collections were obtained from 6 subjects.

DPCP could not be detected in any serum or urine sample from any subject.

DISCUSSION

For the scaled-up extraction method, using 1 ml of urine, the limit of sensitivity of the assay was 20 ng/ml. This is equivalent to 20 μg/l. The maximum 24-h urine output was 2295 ml and the minimum was 805 ml, giving minimum detectable excreted amounts of 16.1 and 45.9 μg. The minimum dose of diphencyprone applied was 0.5 ml of a 1% solution, i.e. at least 5 mg DPCP was applied. The minimum detectable quantity in urine would therefore represent between 0.3 and 0.9% of the administered dose.

Since no DPCP was detected in either serum or urine and less than 1% of the applied dose could have been detected in urine, the results would suggest that DPCP is not absorbed. It is possible that some of the compound is absorbed, rapidly metabolised and either removed from the body by non-renal routes or excreted in urine as unidentified metabolites. However, clearance from serum would have to be exceptionally rapid for no DPCP to be identified at 15 min even when 1 ml of serum was extracted. The evidence would therefore suggest that DPCP is not absorbed through the skin.

Although reassuring as far as it goes, these data provide no guarantees regarding possible toxicity. The only conclusion that can be drawn with some degree of certainty is that DPCP does not accumulate in the body unchanged. It remains possible that the compound is absorbed and metabolised exceptionally rapidly or that breakdown products may be absorbed. Our experience has generally been reassuring, with adverse events being rare and confined to the development of vitiligo in treated areas, severe dermatitis, regional lymphadenopathy and an erythema multiforme-like reaction. However, for the present we believe it remains necessary to explain to patients that knowledge regarding toxicity is limited and to obtain informed consent to the treatment. DPCP should be avoided in pregnancy and used with appropriate caution in female patients of child-bearing age. Further similar work is required to look for breakdown products and metabolites in blood and urine.

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