

INVESTIGATIVE REPORT

A Double-blind Investigation of the Potential Systemic Absorption of Isotretinoin, when Combined with Chemical Sunscreens, Following Topical Application to Patients with Widespread Acne of the Face and Trunk

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This study assessed the systemic absorption of isotretinoin and its metabolites, during a 4-week application of a cream containing 0.1% isotretinoin and chemical sunscreens, compared with a 4% benzoyl peroxide cream, in patients with acne on the face and trunk. Blood was drawn at weeks 0, 1, 2, 3 and 4 and at 96 h post-treatment. Plasma levels of isotretinoin (13-cis-retinoic acid) and tretinoin (all-trans-retinoic acid) were quantified by liquid chromatography with tandem mass spectrometry and the presence of their combined 4-oxo metabolites were monitored from the peak area ratios observed. The isotretinoin group showed no statistically or clinically significant increases in plasma retinoid levels and mean levels did not exceed ± 2 SD of the mean pre-treatment values, indicating that endogenous levels were not being exceeded. No significant differences were detected between the isotretinoin group and the 4% benzoyl peroxide group. These findings indicated that retinoid absorption from a cream containing 0.1% isotretinoin and chemical sunscreens was clinically insignificant, when applied to patients with widespread acne. **Key words:** topical isotretinoin; plasma retinoid levels.

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Acne vulgaris is a chronic skin disease, the initial stage of which involves an impaction of horny cells in the pilosebaceous duct resulting in comedone formation. The closed comedones (whiteheads) can develop into open comedones (blackheads), or rupture to form inflammatory lesions (papules and pustules).

Topical retinoid therapy with 13-cis-retinoic acid (13-cis-RA) is an effective way of treating both inflammatory and non-inflammatory acne (1, 2). 13-Cis-RA isotretinoin is thought to have an anti-comedonal effect, altering the epithelialization of the follicles (3). In animals, topical application of retinoids has been shown to suppress sebum excretion, but this is not so in humans (4).

Owing to the known teratogenic effects of oral retinoids, studies have been conducted to detect the level of absorption, if any, of retinoids from topical formulations. Topical application of 13-cis-RA gel has been shown to result in negligible absorption of the drug (5). In one study, no detectable levels of isotretinoin, or its metabolites, were

identified in blood collected from patients who had applied up to 10 g of 0.05% isotretinoin gel, twice-daily, over a period of 30 days. The assay method used had a detection threshold of 2 ng/ml (6).

A more recent study (7) showed no clinically significant absorption of 13-cis-RA or its metabolites from a gel containing 0.05% isotretinoin combined with 2% erythromycin, or from a cream containing 0.1% isotretinoin. Both of these treatments had been applied to a widespread area of acne affecting the face and trunk, as in the present study.

The current study was performed using a cream that contained 0.1% isotretinoin with added sunscreens. The addition of the sunscreens was aimed at reducing skin irritation from the retinoid as well as being photo-protective. Although the addition of sunscreens was not envisaged to increase the absorption of isotretinoin through the skin in any way, it was thought prudent to investigate any effect that the change in vehicle could have. Flux of isotretinoin has been shown, *in vitro*, to be affected by various vehicles (8) and so this study, which investigated its percutaneous absorption, was performed to address this issue as well as any regulatory concerns that might exist regarding the change in formulation. In order to determine the degree of percutaneous absorption, if any, of the retinoids from the cream, plasma retinoid levels were measured in patients who applied the cream to an extensive area of acne affecting their face and trunk, twice-daily, for 30 days. Tretinoin levels were also measured, as retinoids have a known propensity for photo-isomerization (9). Blood samples were taken before treatment, at weekly intervals throughout the study and at 96 h post-treatment.

MATERIAL AND METHODS

Test medications

Two white creams were tested: 0.1% isotretinoin with chemical sunscreens (IsotrexolTM) and 4% benzoyl peroxide (Solugel^{RS}).

Subjects and treatment application

Patients were recruited into the study from the Department of Dermatology at the Leeds General Infirmary (Table I). Regulatory and Ethics Committee approval had been granted prior to the study commencing and each patient provided voluntary written informed consent before taking part. Patients between 12 and 45 years of age, of any race and either sex, suffering from moderate-to-severe acne vulgaris, affecting the face and trunk, were eligible to enter the study. Patients were not included if they had received oral retinoids during

Table I. Characteristics of treatment groups, including amount of drug applied

Characteristic	Isotrexol cream	Solugel cream
Evaluable patients (n)	13	16
Number of males	8 (62%)	9 (56%)
Mean age (y) ± SD	22.9 ± 7.59	23.4 ± 7.53
Mean height (m) ± SD	1.74 ± 0.11	1.71 ± 0.09
Mean weight (kg) ± SD	64.5 ± 8.6	70.2 ± 13.3
Mean amount of treatment used (g) ± SD	64.6 ± 70.7	103.5 ± 74.7
Mean number of applications ± SD	49.8 ± 5.4	51.4 ± 7.1
Mean surface area covered (cm ²) ± SD	879.7 ± 531.8	1,022.4 ± 488.7
Total amount of treatment used (mg/cm ²) ± SD	68.3 ± 47.2	104.7 ± 57.3
Adverse events ^a	23 in 10 patients	10 in 5 patients

^aAs reported by patients in the safety population (see Results).

the previous year, or if they had used any other acne treatment 1 week prior to the study commencing (a previous study of the same design, using a gel containing 0.05% isotretinoin combined with 2% erythromycin, or a cream containing 0.1% isotretinoin, had shown that plasma retinoid levels returned to baseline within 96 h of stopping treatment (7)).

Female patients were screened for pregnancy and were not entered if they were using anti-androgen contraceptives or were breastfeeding. Thirty-one patients were recruited (15 in the isotretinoin group and 16 in the benzoyl peroxide group) and allocated to one of the two treatment groups. The study was double-blind and both of the treatments were packed in identical white, sealed 40 g tubes.

One week prior to starting treatment (week - 1), patients underwent a medical examination and their medical history and demographic details were recorded. The area of acne on the face, back and chest was measured using planimetry. A blood sample was taken for plasma retinoid analysis. A full blood count and hepatic and renal function tests were also performed.

Patients returned the following week (week 0) to have another blood sample taken for a further plasma retinoid analysis. Patients were dispensed their study medication and instructed to apply it twice-daily (at 12-h intervals) to the affected areas of their face and trunk, following washing. Each patient was dispensed 2 tubes of medication per visit, together with a diary card for them to record when they applied their treatment. Patients were asked to return both tubes at the next visit, regardless of whether they had been used or not.

Patients returned for follow-up visits after 1, 2, 3 and 4 weeks of treatment, and finally 96 h after the cessation of treatment. At each visit, blood was taken for plasma retinoid analysis. At week 4, blood was also taken for a full blood count and hepatic and renal function tests. Details of adverse events and changes in concomitant medication were recorded at each visit.

Patients were instructed to avoid using any other treatment containing a retinoid for the duration of the study. They were also asked not to undergo any UV-light treatment and to minimize their direct exposure to sunlight. In addition, they were requested to limit their alcohol consumption to 14 units per week, to avoid foods with a high vitamin A content (a list of such foods was provided) and to refrain from taking vitamin A or multivitamin supplements.

Estimation of plasma levels of 13-cis-RA and metabolites

All blood samples for retinoid analysis were collected into foil-wrapped disposable syringes, under yellow light, and were immediately placed in an ice bath for a maximum period of

30 min, prior to separation by centrifugation for 10 min at 1,500 g and 4°C.

The resulting plasma was transferred to a labelled, foil-covered polypropylene tube and placed in a container of dry ice, within 15 min, prior to shipment for assay within the same working day. Plasma levels of 13-cis-RA, all-trans-RA and the combined 4-oxo metabolites were determined by using a liquid chromatography-tandem mass spectrometry technique at Covance Laboratories Ltd. (Harrogate, UK) (10). The samples were subjected to minimal preliminary clean-up and analysis was carried out under sodium lighting to prevent photo-degradation and interconversion of the retinoids. As retinoids are very unstable, great care was taken during this study to ensure that no degradation or interconversion occurred during sample processing, because this could potentially have led to an underestimate of the plasma levels. An internal standard, acitretin, was added at the start of the extraction and was used in the calculation of the plasma levels of isotretinoin and tretinoin.

The lower limit of quantification was 0.73 ng/ml for 13-cis-RA and 0.72 ng/ml for all-trans RA. The limits of detection were 0.35 and 0.34 ng/ml, respectively. Because the 4-oxo metabolites of 13-cis RA and all-trans RA co-elute, the combined 4-oxo metabolites in the plasma samples were monitored from the observed peak area ratios of the combined 4-oxo metabolites peak to the internal standard (not quantified).

Recording of adverse events

Adverse events reported by patients were classified into body systems and preferred terms using COSTART (coding symbols thesaurus of adverse reaction terminology).

Data analysis

Ninety-five percent confidence intervals for the magnitude of change of plasma levels from start of treatment (week 0) to weeks 1, 2, 3 and 4 and 96 h post-treatment were calculated. Those that did not span zero were taken to indicate a significant difference. One patient, who presented with high plasma levels of isotretinoin and combined 4-oxo metabolites at week 0, was excluded from the analysis before application of study medication. Table II displays the changes in mean plasma retinoid concentrations from start of treatment (week 0), without these outlying data points.

RESULTS

Characteristics of the treatment groups for the main study

Thirty-one patients were recruited, of whom 30 were evaluable for safety analysis: one patient withdrew after the first blood sampling and did not, therefore, receive any trial medication.

The two treatment groups were reasonably well-matched in terms of age, sex, height, weight and race. In addition, there were no obvious differences between the groups with regard to concomitant medications taken throughout the study. However, the mean weight of study medication used was higher in the 4% benzoyl peroxide group, reflecting the greater mean total area of acne that was treated.

Comparison of plasma retinoid levels

Mean plasma 13-cis-RA ranged between 1.79 and 2.18 ng/ml in the isotretinoin group and between 1.51 and 1.73 ng/ml in the 4% benzoyl peroxide group. Mean plasma all-trans-RA (tretinoin) ranged between 1.28 and 1.40 ng/ml in the isotretinoin group and between 1.29 and 1.51 ng/ml in the

Table II. Change in mean plasma retinoid concentrations following topical application of the 2 test formulations

	Isotrexol cream	Solugel cream
<i>Isotretinoin (ng/ml)</i>		
Week 1	0.19 ± 0.60 ^a	- 0.15 ± 0.37
Week 2	0.09 ± 0.42 ^a	- 0.13 ± 0.25
Week 3	- 0.04 ± 0.51 ^a	- 0.11 ± 0.52
Week 4	0.04 ± 0.38 ^a	- 0.03 ± 0.37
Week 4+ 96 h	- 0.15 ± 0.53	- 0.15 ± 0.17
<i>Tretinoin (ng/ml)</i>		
Week 1	- 0.02 ± 0.25	- 0.08 ± 0.28
Week 2	0.04 ± 0.39	- 0.16 ± 0.28
Week 3	- 0.05 ± 0.32	- 0.14 ± 0.39
Week 4	0.06 ± 0.25	0.02 ± 0.23
Week 4+ 96 h	0.01 ± 0.31	- 0.22 ± 0.34
<i>Combined 4-oxo metabolites: expressed as difference in peak area ratios</i>		
Week 1	0.02 ± 0.04 ^a	0.00 ± 0.02
Week 2	0.00 ± 0.03 ^a	0.00 ± 0.02
Week 3	0.01 ± 0.03 ^a	0.02 ± 0.03
Week 4	0.02 ± 0.03 ^a	0.02 ± 0.02
Week 4+ 96 h	0.01 ± 0.05	0.02 ± 0.05

^aAn outlier was omitted when computing each of these data points.

4% benzoyl peroxide group. Mean plasma 4-oxo metabolites peak area ratio ranged between 0.08 and 0.11 in the isotretinoin group and between 0.05 and 0.08 in the 4% benzoyl peroxide group.

There were no significant differences observed in 13-cis-RA, all-trans-RA or 4-oxo metabolite plasma levels between the isotretinoin and 4% benzoyl peroxide groups. During the course of the study, mean 13-cis-RA, all-trans-RA and 4-oxo metabolite plasma levels remained within 2 SD of the pre-treatment levels, indicating that no clinically significant changes had occurred.

Adverse events

No serious adverse events were reported throughout the study and no patients withdrew as a result of an adverse event.

In both treatment groups the most common adverse events involved the skin. The incidence of these skin reactions was higher in the isotretinoin group (18 events reported by 9 patients) than in the 4% benzoyl peroxide group (6 events reported by 2 patients). For all other adverse events, the profile was similar for both groups. Both treatments had similar tolerance ratings at weeks 2 and 4.

DISCUSSION

This study was designed to simulate clinical use of a topical acne treatment containing a retinoid, which is applied to a large area of skin, *i.e.* maximized use. Its aim was to determine any systemic absorption of 13-cis-RA and its metabolites from a cream containing 0.1% 13-cis-RA with chemical sunscreens, indicated for the treatment of acne vulgaris. A previous study on Isotrex[®] cream, containing 0.1% 13-cis-RA, but no sunscreens, showed no clinically significant retinoid absorption (7).

Systemic absorption of ingredients contained in topical

treatments must always be considered, as systemic effects may be undesirable. This is particularly the case with retinoids with their known teratogenic potential, effect on the liver enzyme profile and, more recently, their suspected link with depression. In this study, the mean amount of the isotretinoin cream applied by the patients (64.6 g over 4 weeks) is at the upper limit of what would be expected to be applied in routine clinical practice (11).

At no time were any statistically or clinically significant increases in plasma retinoid levels detected in any of the patients participating in this study. There were no significant differences observed in 13-cis-RA, all-trans-RA or 4-oxo metabolite plasma levels between the isotretinoin and 4% benzoyl peroxide groups.

This finding confirms that the absorption of retinoids from the isotretinoin cream was negligible, as the 4% benzoyl peroxide cream does not contain retinoids. During the course of the study, mean 13-cis-RA, all-trans-RA and 4-oxo metabolite plasma levels remained within 2 SD of the pre-treatment levels. This indicates that the variation measured was well within the range of normal endogenous levels (levels of 13-cis-RA range from <1 to 2.5 ng/ml and those of all-trans-RA from <1 to 2 ng/ml (12)) and that no clinically significant changes had occurred. This study could have missed peak elevations of retinoids, but does show no absorption of topical retinoids.

Although both treatments were similarly tolerated, the incidence of adverse events affecting the skin was higher in the isotretinoin group. The majority of these adverse events involved dry skin, pruritus and rash and were short-lived. They were never classified as serious and did not cause the patients to withdraw from the study.

Plasma levels can be changed by a variety of factors. For example, repeated ingestion of vitamin A (0.25 mg retinol equivalents/kg) has been reported to increase the endogenous level of all-trans-RA by a factor of 6 (13). However, the results of this study are in keeping with others, showing that repeated and maximized topical application of the isotretinoin cream, containing 0.1% 13-cis-RA, results in negligible changes in the plasma retinoid levels. In addition, the lack of any difference between the isotretinoin and 4% benzoyl peroxide groups indicates that there was no clinically significant absorption of retinoids from isotretinoin cream.

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