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Effect of Topical Application of 13-Cis Retinoic Acid on Skin of Hairless Rats and Hairless Mice*

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Summary. The potential effectiveness of topical 13-cis retinoic acid (13-cis RA) as a sebosuppressive agent was evaluated in hairless ("fuzzy") rats and hairless mice. At nontoxic dosages (i.e., concentrations which induced no weight loss), topical 13-cis RA had no detectable sebosuppressive effects in either of these species. In hairless rats, the topical application of 0.2% 13-cis RA induced more severe symptoms of toxicity than was induced by the administration of equivalent amounts of the drug by either oral or subcutaneous routes. Due to variability in species sensitivity to 13-cis RA, the potential effectiveness of the topical use of this retinoid can probably only be evaluated in human volunteers.

Key words: 13-Cis retinoic acid – Hairless rats – Hairless mice – Sebum production – Epidermis

Introduction

13-Cis retinoic acid (13-cis RA; isotretinoin, Accutane) is available commercially as an oral preparation for the treatment of nodulocytic acne. The effectiveness of this drug for the treatment of severe acne has been convincingly demonstrated in numerous clinical studies [6, 13, 14]. Indeed, 13-cis RA appears to be ideally suited for treating acne, since many of its effects, such as reversal of abnormal keratinization [2, 7], its anti-inflammatory properties [16], and particularly its effect of suppressing sebaceous gland lipogenesis [9, 19], are remarkably targeted to the pathogenic processes involved in acne. Oral administration of 13-cis RA is not without some side effects, and for that reason, there has been interest in its use as a topical preparation. It would be reassuring to have some evidence of its potential effectiveness before attempting this method of administration in humans. Unfortunately, to date, there have been few reports of the topical use of 13-cis RA in animal models.

In studies of the histological changes in rhino-mouse skin following the topical application of a number of retinoids, Ashton et al. [1] and Mezick et al. [11, 12] included observations on the topical use of 13-cis RA. The prime focus of these studies was to compare the antikeratinizing effect of different retinoids by measuring the reduction in size of the keratinized utriculi characteristically present in the skin of rhino mice. Both groups of investigators found that in this model, 0.1% solutions of 13-cis RA were effective topically as antikeratinizing agents, although all-trans retinoic acid was considerably more effective in this respect.

In preliminary reports, Plewig et al. [17, 18] studied the effect of topical 13-cis RA on the histologic size of sebaceous glands following its application to hamster flank organs and hamster ear lobes, and found it to have a sebosuppressive effect in this model.

In the present report we describe our experiments with topical administrations of 13-cis RA to hairless Sprague-Dawley rats and Skh:HR-1 hairless mice.

Materials and Methods

Animals

Hairless ("fuzzy") mutants of Sprague-Dawley rats were obtained from Dr. L. Maxwell (Bristol Laboratories, Hillside, NJ, USA) and bred in our own laboratories. Female hairless Skh:HR-1 mice were purchased from the Skin and Cancer Hospital, Temple University Philadelphia, Pennsylvania.

Both male and female rats were used, but for each specific experiment the animals used were of the same gender and age (11 – 12 weeks). Animals were housed individually and fed standard laboratory chow ad libitum. While being treated, they wore

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plastic collars which prevented ingestion of the topically applied medication.

Drug

13-Cis RA (a gift from Dr. W. E. Scott, Hoffman-LaRoche, Nutley, NJ, USA) was maintained at −60° C in amber vials and was emulsified undiluted at appropriate concentrations in a lipid base made according to a previously recommended formulation [8] consisting of 75.3% soybean oil, 19.4% vegetable shortening, 4.8% beeswax, 0.3% ethylenediaminetetraacetate (EDTA), and 0.07% butylated hydroxytoluene. The emulsions were made in semidarkness and, once prepared, were maintained in the dark at −60° C. Prior to use, the emulsions were gently warmed in a water bath to bring them to a viscous consistency. The lipid base to which no 13-cis RA had been added was applied as a negative control.

Treatment

The animals were treated once daily (three to ten animals per group) for 5 days per week over 4-week periods. The rats and mice were treated with 0.5 and 0.1 ml of appropriate emulsions, respectively; these were spread over the skin of the entire back using 1-ml plastic syringes. Four concentrations (1.0%, 0.2%, 0.02%, and 0.01%) of 13-cis-RA emulsion were tested.

Evaluations

Weight of Animals. The animals were weighed before treatment and once weekly thereafter.

Sebaceous-gland Size and Lipogenesis. In vitro lipid synthesis of the skin was assayed according to the methods described by Cooper et al. [4]. Two 3-mm punch biopsies of dorsal skin obtained from each animal at the completion of treatment were incubated in Krebs-Ringer phosphate buffer (pH 7.4) containing antibiotics and 5 μCi D-U-^{14}C-dextrose per milliliter (sp. act., 302 mCi/mmol). Incubations were carried out in air: 5% CO_2 in a metabolyte shaking water bath at 37° C for 3 h. After incubation, the biopsies were rinsed, the dermis and epidermis were separated and homogenized, and the total lipids were extracted from both using the extraction procedure of Bligh and Dyer [3]. The radioactivity in the total lipid fraction was used to express in vitro lipid synthesis per biopsy.

The animals in all experiments were biopsied before, during, and at the completion of treatment. The biopsies were fixed in 10% formalin, and serial longitudinal sections were stained with eosin and hematoxylin according to standard procedures [10].

Results

Hairless Rats

In the initial studies of rats treated with 1.0% and 0.2% 13-cis RA, toxic effects (wobbliness, weight loss) were manifested within 72 h of the initial application of 13-cis RA. By 96 h, a dark, scaly dermatitis had developed over the skin of the backs. After four applications, the animals were emaciated and clearly ill. The treatments were stopped, but rats treated with the 1.0% emulsion died on the 5th day. The total dose of 13-cis RA applied had been 20 mg. Skin changes consisted of thick, brown, cracked, scaly epidermis over the entire body, including the abdomen, legs, and face. In some areas, this had sloughed off and eroded, exposing oozing, hyperkeratotic brown skin beneath it. The tail and ears were only slightly affected (Fig. 1).

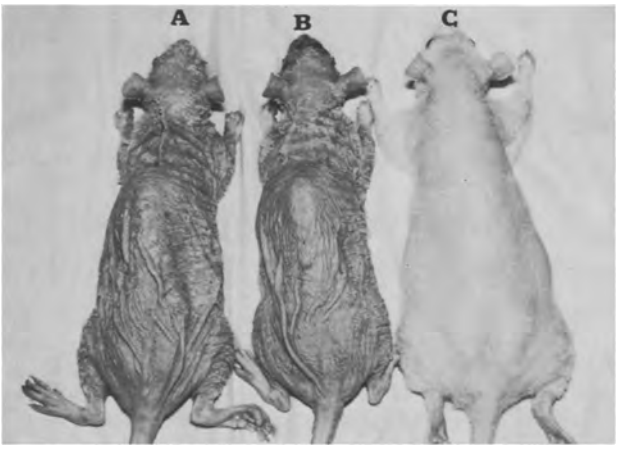

Fig. 1. Appearance of hairless rats after 4 days of topical application of a total of 20 mg 13-cis RA (**A**), 4 mg 13-cis RA (**B**), and lipid base without 13-cis RA (**C**)

The rats treated with 0.2% 13-cis RA showed identical changes, including skin changes, weight loss, and wobbliness, after having received a total of only 4 mg 13-cis RA over a 4-day period. At necropsy, no gross lesions were observed. Skin biopsies demonstrated marked hyperkeratosis and subcorneal inflammation.

Since these toxic effects of the topical administration of 4 mg 13-cis RA were more severe than had been anticipated on the basis of toxicologic studies of systemic administration of 13-cis RA in haired rats, 13-cis RA was administered to hairless rats using oral and subcutaneous routes. Four rats were fed 1 mg 13-cis RA emulsified in peanut butter, and four were subcutaneously injected with 1 mg 13-cis RA daily for 4 days. No overt manifestations of toxicity were observed. The skin appeared normal. However, the animals did demonstrate an average weight loss of 4.2%. Thus, it appeared that the hairless mutants may be more acutely sensitive to toxic effects of 13-cis RA than haired variants. Rats treated with lower doses (eight rats per group) of 0.01% and 0.02% 13-cis RA exhibited no overt signs of toxicity. After 4 weeks of application (i.e., total of 1 and 2 mg 13-cis RA), all animals seemed to be healthy. A comparison of weights indicated that the control animals and the animals on the lower dosage had an average weight gain of 7.0%, but animals treated with 0.02% 13-cis RA experienced no weight gain (Table 1).

Gross changes developed in the skin of five of the eight rats treated with the 0.02% emulsion of 13-cis

Table 1. Weight of rats treated with vehicle or with 0.02% 13-cis RA over a 4-week period

Treatment groups[a]	Weight (g)[b]			
	Baseline	7 days	14 days	28 days
Controls	230± 9	239± 8	237± 6	246± 6
0.02% 13-cis RA	223±20	221±19	221±18	224±21

[a] Eight rats per group
[b] Mean for eight rats per group ± SD

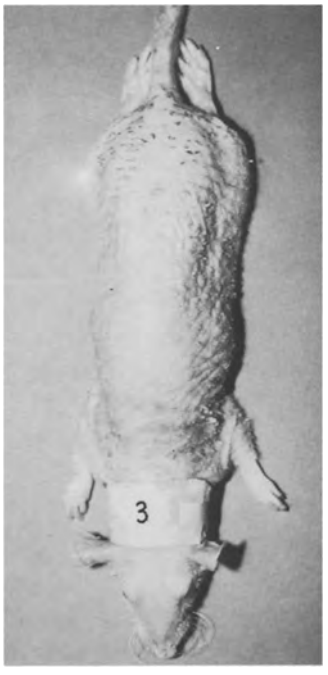

Fig. 2. Male rat treated with a total of 2 mg (0.1 mg/day) 13-cis RA over a 4-week period

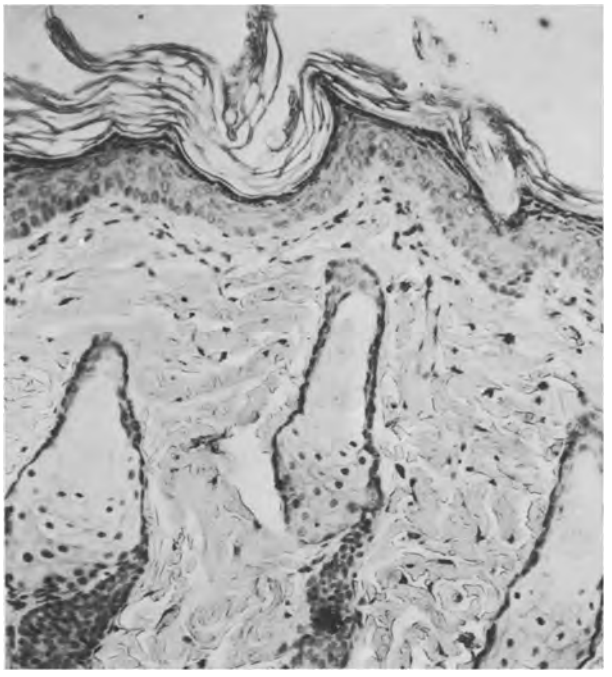

Fig. 3. Cross section of skin of the rat shown in Fig. 2. Note the presence of large sebaceous glands

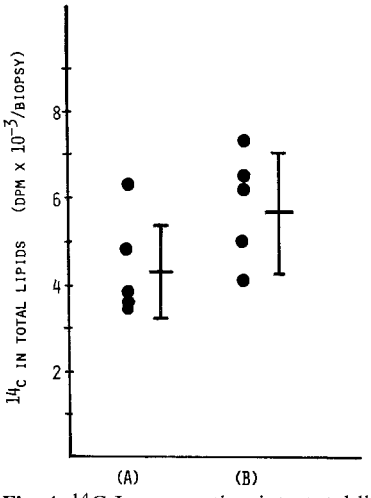

Fig. 4. ^{14}C Incorporation into total lipid in skin biopsies from rats treated topically for 4 weeks with 0.02% 13-cis RA (**A**), and rats treated for 4 weeks with lipid base alone (**B**). The results are presented as the average amount of ^{14}C incorporated by two biopsies per rat and as means ± standard deviations. The differences in mean values was not significant ($P > 0.20$)

RA. These changes consisted of brown scaliness over the entire body, but particularly over the dorsal areas (Fig. 2). Histologically the skin of the treated animals was hyperkeratotic, but the sebaceous glands were similar in size to the glands in pretreated skin and in the skin of the control animals (Fig. 3).

The results of the in vitro lipid biosynthesis studies are summarized in Fig. 4. The difference in the mean values of the treated animals and the control group was not significant (Student t-test, $P > 0.20$).

Hairless Mice

Eight out of the twenty mice treated with 13-cis RA (0.01% and 0.02% emulsions) experienced weight loss and developed brown scaliness over the skin of the entire body. These changes were unrelated to the weight of the animals at the beginning of treatment and occurred in animals treated with the 0.01% emulsion as well as with the 0.02% emulsion.

Histologic evaluations of sebaceous-gland size failed to demonstrate differences in gland size in the skin of the 13-cis-RA-treated animals and the controls (Fig. 5).

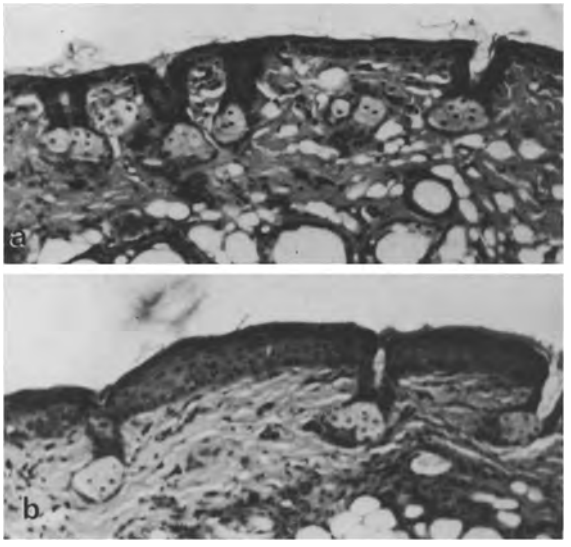

Fig. 5. a Cross section of untreated hairless-mouse skin. **b** Cross section of hairless-mouse skin following treatment with 0.01% 13-cis RA for 4 weeks. Note that despite the change in cutaneous morphology, the size of the sebaceous glands remains similar

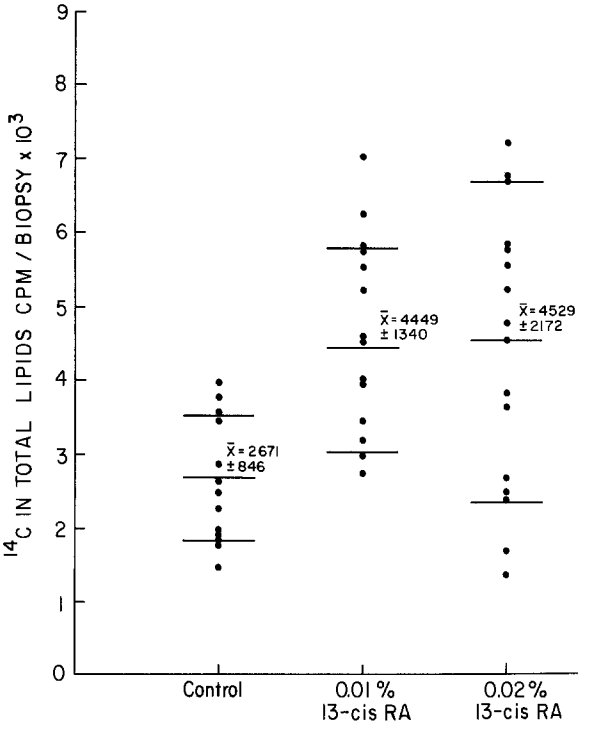

Fig. 6. ^{14}C Incorporation into total lipids extracted from skin biopsies of hairless mice treated topically for 4 weeks with lipid vehicle, 0.01% 13-cis RA, and 0.02% 13-cis RA. The results are presented as mean cpm per biopsy per treatment group. The differences in mean values between the 13-cis-RA-treated animals and the controls were significant ($P > 0.01$)

The results of the ^{14}C-glucose-incorporation studies are summarized in Fig. 6. The mean ^{14}C-glucose incorporation was significantly higher in the skin of animals treated both with 0.01% and with 0.02% 13-cis RA than in that of the controls. Follow-up studies to analyse this unexpected finding demonstrated that increases in radiolabeled lipid synthesis occurred in the epidermis as well as in the dermis. Thin-layer chromatographic analysis of the different lipid classes using the solvent system recommended by Downing [5] indicated that the primary increase in ^{14}C-glucose incorporation in 13-cis-RA-treated animals was in the triglyceride fraction.

Discussion

These essentially negative experiments demonstrated that the topical application of 13-cis RA is not effective at suppressing sebaceous lipogenesis in hairless-rat or -mouse skin, at least during the 4-week treatment periods used in the present study.

They also demonstrated the great variability in species sensitivity to the toxic effects of retinoids. According to Gomez and Moskowitz [8], hamsters have survived intraperitoneal injections of a total of 144 mg 13-cis RA. In other studies, rhino mice have tolerated the topical administration of 1 mg 13-cis RA over a 2-week period [1, 11, 12], and hamsters have tolerated 15 days of topical treatment with 0.3% emulsions [18]. However, in the present study rats weighing 10–12 times more than rhino mice exhibited cutaneous changes and, in some instances, weight loss at cumulative doses of as little as 2 mg 13-cis RA administered topically over a 4-week period. A total of 4 mg 13-cis RA applied topically over 4 days induced acute toxic effects.

In unrelated studies, we have fed Wistar rats (haired) 13-cis RA (obtained from Hoffman-LaRoche in gelatinized pellet form containing 11% of the active drug) at doses of 0.5 and 3 mg/kg body weight per day, so that they received a total of 2 and 12 mg 13-cis RA over a 2-week period. In such studies, the animals exhibited no weight loss.

13-Cis RA is recognized as a labile isomer which is partially transformed to all-trans RA on exposure to light. For this reason, it is difficult to compare the concentration of active drug in studies which have used different solvent systems and different routes of administration. The solubilization of 13-cis RA in a lipid base containing antioxidants probably increases the stability and percutaneous penetration of the drug (Dr. A. Golberg, Hoffman-LaRoche, personal communication). This may partially explain why, in the present study, small doses were so effective in inducing symptoms of hypervitaminosis.

Human, sebaceous glands are sensitive to suppression caused by the oral administration of 13-cis

RA. In rats and mice, sebaceous gland suppression did not occur even at doses which induced systemic toxic effects manifested by weight loss.

At this point, we cannot explain the increase in ^{14}C-glucose incorporation into cutaneous lipids in the 13-cis-RA-treated hairless mice. The fact that epidermal, as well as dermal lipid synthesis was significantly increased suggests that the change in lipid metabolism was nonspecific and probably unrelated to sebaceous gland function. Histologically, there was no evidence of change in the size of the sebaceous glands of the treated animals. Ashton et al. [1] have reported that in their studies with rhino mice, the sebaceous glands appeared to be larger following topical retinoid treatment. However, they pointed out that the reduction in size of the horn-filled utricles resulted in the illusion of an increase in size of the sebaceous glands and that, in reality, the size of the sebaceous glands in this model remained unchanged.

It is clear from results of Plewig et al. [15–18] and those of the present study that the sensitivity of sebaceous glands to 13-cis RA varies greatly with different species. In humans, these glands are uniquely sensitive. To date, the sebaceous glands of laboratory animals (other than perhaps the hamster) have not been found to share this sensitivity. For this reason, it may be impossible to evaluate the effect of the topical application of 13-cis RA in anything other than the human model.

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References

1. Ashton RE, Connor MJ, Lowe NJ (1984) Histologic changes in the skin of the rhino mouse (hr^{rh}/hr^{rh}) induced by retinoids. J Invest Dermatol 82:632–635
2. Baden HP, Buxman MM, Weinstein GD, Yoder FW (1982) Treatment of ichthyosis with isotretinoin. J Am Acad Dermatol 6:716–720
3. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:311–317
4. Cooper MF, McGrath E, Shuster S (1976) Sebaceous gland lipogenesis in human skin. Br J Dermatol 94:165–172
5. Downing DT (1968) Photodensitometry in the thin-layer chromatographic analysis of neutral lipids. J Chromatogr 28:91–99
6. Farrell LN, Strauss JS, Stranieri AM (1980) The treatment of severe cystic acne with 13-cis retinoic acid. J Am Acad Dermatol 3:602–611
7. Goldsmith LA, Weinrich AE, Shupack J (1982) Pityriasis rubra pilaris response to 13-cis retinoic acid (isotretinoin). J Am Acad Dermatol 6:710–715
8. Gomez EC, Moskowitz RJ (1980) Effect of 13-cis retinoic acid on the hamster flank organ. J Invest Dermatol 74: 392–397
9. Landthaler M, Kummermehr J, Wagner A, Plewig G (1980) Inhibitory effects of 13-cis retinoic acid on human sebaceous glands. Arch Dermatol Res 69:297–309
10. Lillie RD (1954) Histopathologic techniques and practical histochemistry. Blakiston, New York Toronto, pp 114–115
11. Mezick JA, Chabria MC, Thorne EG (1982) Topical and oral (p.o.) effects of 13-cis retinoic acid (13-cis RA) and all-trans-retinoic acid (RA) on horn-filled utriculi (pseudocomedones) size in the rhino mouse. J Invest Dermatol 78:350
12. Mezick JA, Bhatia MC, Capetola FJ (1984) Topical and systemic effects of retinoids on horn-filled utriculus size in the rhino mouse. A model to quantify "anti-keratinizing" effects of retinoids. J Invest Dermatol 83:110–113
13. Peck GL, Olsen TG, Yoder FW, Strauss JS, Downing DT, Pandya M, Butkus D, Arnaud-Battandier J (1979) Prolonged remissions of cystic and conglobate acne with 13-cis retinoic acid. N Eng J Med 300:329–333
14. Peck GL, Olsen TG, Butkus D, Pandya M, Arnaud-Battandier J, Gross EG, Windhorst DB, Cheripko J (1982) Isotretinoin versus placebo in the treatment of cystic acne. J Am Dermatol 6:735–745
15. Plewig G (1980) Der Einfluß der aromatischen Retinoids Ro 10-9359 und der 13-cis Retinsäure Ro 4-3780 auf die Talgdrüsen des Syrischen Hamsters. Arch Dermatol Res 268:239–246
16. Plewig G, Wagner A (1981) Anti-inflammatory effects of 13-cis retinoic acid. An in vivo study. Arch Dermatol Res 270:89–94
17. Plewig G, Wagner A, Nikolowski J, Landthaler M (1981) Effects of two retinoids in animal experiments and after clinical application in acne patients: 13-cis retinoic acid, Ro 4-3780, and aromatic retinoid, Ro 10-9359. In: Orafanos CE, Braun-Falco O, Farber EM, Grupier C, Polano MK, Schuppli R (eds) Retinoids. Springer, Berlin Heidelberg New York, pp 219–235
18. Plewig G, Ruhfus A, Klövekorn W (1983) Sebum suppression after topical application of retinoids (arotenoid and isotretinoin). J Invest Dermatol 80:357
19. Strauss JS, Stranieri AM, Farrell LN, Downing DT (1980) The effect of marked inhibition of sebum production with 13-cis retinoic acid on skin-surface lipid composition. J Invest Dermatol 74:66–67

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