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The Pharmacology of a Novel Topical Retinoid, BMY 30123: Comparison with Tretinoin

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Abstract—Preclinical studies pertaining to the pharmacology and toxicology of BMY 30123 (4-acetamidophenyl retinoate) are reported. BMY 30123 is a novel compound which has topical retinoid activity. This compound exhibits lower toxicity, both local and systemic, than other clinically used topical retinoids such as tretinoin (all-*trans* retinoic acid) in animal models. BMY 30123 is effective in a number of retinoid sensitive skin models including the rhino mouse utriculi reduction assay, the mouse epidermal hyperplasia model and in the suppression of DNA synthesis in mouse skin stimulated with phorbol ester. BMY 30123 was equipotent with tretinoin in these topical models. In the rhino mouse model the ED30 values for BMY 30123 and tretinoin were 0·037 and 0·015 mM, respectively. In addition, BMY 30123 was active in the UVB-induced photodamaged mouse model, another retinoid sensitive model. One of the problems associated with topically applied tretinoin is local irritation. Therefore, for topical therapy to be optimal, it is important to reduce or minimize local irritation. Repeated applications of BMY 30123 to rabbit skin resulted in low skin irritation. The first perceptible signs of skin irritation produced by BMY 30123 occurred at a dose 10 times higher than that observed for tretinoin. BMY 30123 also exhibits low retinoid activity after oral or i.p. administration in mice and produced no signs of hypervitaminosis A-related toxicity at twenty times the no effect dose of tretinoin. Because retinoids are effective modulators of epidermal growth and differentiation, this compound should be useful for the treatment of cutaneous disorders that exhibit altered epidermal differentiation such as acne, psoriasis, ichthyosis and epithelial tumours. While BMY 30123 and tretinoin are shown here to be equipotent in animal efficacy models, the low skin irritation activity gives BMY 30123, at a minimum, a 10-fold enhancement in the therapeutic index relative to tretinoin and suggests that retinoid efficacy and skin irritation are separable phenomena.

Topically applied retinoids have potential therapeutic utility in the treatment of various dermatological disorders such as acne, psoriasis, hyperkeratosis and in the prevention of epithelial cancers (Moon & Itri 1984; Peck 1984). In addition, topical tretinoin (all-*trans* retinoic acid) is reported to accelerate the repair of photodamaged human skin (Kligman et al 1986; Weiss et al 1988). The anti-skin wrinkling activity observed for this retinoid has drawn attention to this class of compounds and has led to an expansion in retinoid pharmacology research.

Recently, three nuclear receptors specific for retinoic acid have been identified, RARα, -β, -γ (Brand et al 1988; Krust et al 1989). Of these three, RARγ is the predominant form in the skin (Krust et al 1989). Isoforms of these receptor proteins have also been reported (Kastner et al 1990; Leroy et al 1991; Zelent et al 1991) and their presence suggests a molecular mechanism explaining the wide diversity of biological activity seen with the use of retinoids.

Most synthetic retinoids, like their parent compound retinol (vitamin A), have a broad spectrum of side effects, especially when dosed systemically. These side effects of retinoids have collectively been described as the hypervitaminosis A syndrome. Mucocutaneous reactions are the most frequently reported hypervitaminosis A-related side effects. Other hypervitaminosis A symptoms include ophthalmic changes, blood lipid changes, hepatotoxic effects, skeletal hyperostosis and teratogenic effects (Yob & Pochi 1987). Most of these side effects can probably be avoided by the topical application of retinoids, as demonstrated for tretinoin. Topical therapy is expected to have an advantage over systemic use since it maximizes the cutaneous drug concentration and minimizes systemic exposures, thereby reducing the potential for systemic drug side effects.

One of the drawbacks associated with topically applied tretinoin is local irritation. For topical therapy to be optimal, it is also important to reduce or minimize local irritation. Our objective has been to identify topically active retinoids with potency equal to or better than tretinoin but with less local irritation and negligible risk of systemic toxicity.

BMY 30123, 4-acetamidophenyl retinoate, is a novel topical retinoid which meets the above stated objective (Fig. 1). This report describes the pharmacology of BMY 30123 which has potent in-vivo retinoid activity in the skin, and possesses low skin irritation potential. In all experiments we have compared the activity of BMY 30123 with tretinoin which is the active component in a widely used topical anti-acne preparation.

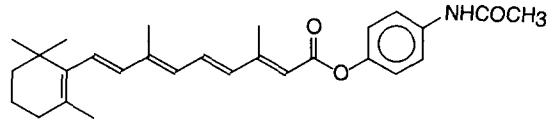

FIG. 1. Structure of BMY 30123.

Materials and Methods

Materials

Tretinoin was purchased from Eastman Kodak Company

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(Rochester, NY, USA). BMY 30123 was prepared by Eastman Kodak Company (Rochester, NY, USA). 12-*O*-Tetra-decanoylphorbol-13-acetate (TPA) was obtained from Sigma Chemical Company (St Louis, MO, USA). [Methyl-^{3}H]Thymidine was purchased from DuPont/NEN (Boston, MA, USA). Bacterial collagenase (CLSPA grade, from *Clostridium histolyticum*) was obtained from Worthington Biochemical Corporation (Freehold, NJ, USA). This collagenase was further purified by chromatography on Sephacryl S200 HR gel (Pharmacia, Uppsala, Sweden). Azocoll (< 50 mesh) was purchased from Calbiochem (La Jolla, CA, USA). The streptomycin/penicillin mixture used in the collagen labelling buffer was from Flow Laboratories (McLean, VA, USA). Krebs-Ringer buffer and all other buffer supplements including ascorbic acid and protease inhibitors were from Sigma (St Louis, MO, USA). For precipitation of the collagenase-digested samples, tannic acid and trichloroacetic acid were also obtained from Sigma. L-[2,3-^{3}H]Proline, [methyl-^{14}C]methylated myosin and L-[5-^{3}H]tryptophan were from New England Nuclear (Boston, MA, USA). The skin replicas were prepared from Silfo Dental Impression Material (Flexico Developments Ltd, Potters Bar, UK).

Animals

Nine- to fourteen-week old female hairless rhino mice (hr^{rh} hr^{rh}) were purchased from the Skin and Cancer Hospital, Temple University Health Sciences Center (Philadelphia, PA, USA). Hairless mice (HRS/J), age 6- to 8-weeks, were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and used in the epidermal DNA synthesis experiments. For the mouse photodamage model, hairless mice SKH-1 (hr/hr) were obtained from Charles River (Wilmington, MA, USA). CD-1 mice were purchased from Charles River. Male New Zealand White rabbits, 10- to 12-weeks old, were purchased from Beckens Farms (Sanborn, NY, USA). Animals had free access to food and water and were kept on a 12 h light:dark cycle. The animal rooms were fitted with yellow lights to minimize retinoid photoisomerization.

Rhino mouse utricle model

Utricle diameter of rhino mouse skin was assessed in whole mounts of epidermal sheets, as described previously (Mezick et al 1984). Female hairless rhino mice (hr^{rh}hr^{rh}) were treated with the anti-parasitic agent, trichlorfon (Combot), administered in their drinking water for 2 weeks to eliminate pinworms. An additional 2 weeks were allowed to pass without Combot treatment before mice were used in this study. Retinoids were applied once daily, 5 days per week, for one week. Three days after the last treatment, the animals were killed by CO_2 inhalation. A 7/8″ diameter circular area of treated skin was removed and dissected in half. The epidermis from one-half of the biopsy was separated from the dermis after incubation of skin in 0·5% acetic acid for 10–20 h at 4°C. These epidermal sheets were fixed in formalin, dehydrated with ethanol, and cleared in xylene. The other half of the biopsy was placed in 10% buffered formalin and sent for processing and preparation of haematoxylin- and eosin (H&E)-stained vertical sections. To assess utricle diameter, each epidermal sheet was placed on a glass slide in a few drops of xylene. The diameter of 40 utricles was measured in each epidermal specimen with an image analyser (Image Measure, Microscience, Federal Way, WA, USA).

Phorbol ester-stimulated epidermal DNA synthesis in hairless mice

Male or female hairless mice (HRS/J) were 6- to 8-weeks old at the time of study. DNA synthesis was measured by [^{3}H]thymidine incorporation into epidermal DNA. Mice were given 25 μCi of [^{3}H]thymidine by intraperitoneal injection 14 h after TPA was applied and killed 1 h later. The skin was removed and placed on ice, epidermal side down. Epidermis was removed from a 3/4″ diameter circular area (scored with an arch punch) by gentle scraping, after immersion of skin in 55°C water for 1 min. Epidermal samples were then immediately frozen in liquid nitrogen. DNA was extracted by a modified Schneider procedure as described previously (Otani et al 1980). Epidermis was homogenized with a Brinkman Polytron in 2·0 mL of ice-cold 0·5 M perchloric acid (PCA). Acid insoluble material was precipitated by centrifugation (800 *g* at 4°C) and washed twice with 0·5 M PCA. DNA was hydrolysed by resuspension of the pellet in 1·5 mL of 0·5 M PCA and heating at 90°C for 30 min. Tubes were chilled on ice and the precipitate sedimented by centrifuging at 800 *g*. The supernatant was saved and the precipitate discarded. Duplicate 0·5 mL aliquots of the acid hydrolysate from each sample were placed in glass scintillation vials, 10 mL of Insta-Gel added, and counted in a liquid scintillation counter. A 0·2 mL portion of the acid hydrolysate from each sample was used to determine total DNA content by the colorimetric method of Burton (1956) modified according to Gendimenico et al (1988).

Retinoid-induced hyperplasia in hairless mice

Hairless mice were treated with retinoids on day 0 and day 1 as previously described (Connor et al 1986). The resulting epidermal hyperplasia is maximal on days 3–5. For convenience, the reported studies were carried out with the animals being killed on day 4. A 7/8″ punch biopsy was taken and a 1/4″ × 1″ rectangular strip of skin was dissected and placed in buffered formalin. The skin was processed and vertical sections stained with H&E were prepared. The epidermal thickness was quantitated at 15 interfollicular areas of each section with an image analyser (Image Measure, Microscience, Federal Way, WA, USA). BMY 30123 was compared with tretinoin for its ability to induce epidermal hyperplasia.

Repair of UV-induced dermal damage in hairless mice

Six- to eight-week old female hairless mice (SKH-1) were used in this study. Animals were divided into irradiated and non-irradiated groups. In the irradiated group, increasing amount of UVB was applied for 10 weeks, three times a week on Monday, Wednesday and Friday, using a bank of eight Westinghouse FS-40 sunlamps placed 16 cm above the back of the mice. During the first four weeks, the UV radiation dose per exposure was progressively increased from one minimal erythemal dose (MED) to four MEDs. The 4-MED dose per exposure was then continued for the remaining six weeks (total dose = 1·4 J cm^{-2}). After the termination of UVB exposure, 50 μL of various concentrations of BMY

30123 or tretinoin in ethanol was applied 5 times a week for 10 weeks to the dorsal skin of both irradiated and non-irradiated animals. At week 2, 4, 6, 8 and 10 of treatment, animals were killed. Six 5/16″ punches were taken from the skin of each animal. After removal of the sub-cutaneous fat tissue, each punch was rinsed in 70% ethanol followed by modified Krebs-Ringer buffer (Krebs-Ringer buffer supplemented with 15 mM sodium bicarbonate, 50 μg mL^{-1} ascorbic acid, 100 μg mL^{-1} β-aminopropionitrile, 50 μg mL^{-1} streptomycin, 500 units mL^{-1} penicillin, 20 mM HEPES and 30 mM glucose, pH 7·2). Punches were pressed dry with filter paper before weighing, then labelled with 20 μCi of [2,3-^{3}H]proline in 1 mL of modified Krebs-Ringer buffer for 2 h at 37°C. The reaction was terminated by freezing the samples at −80°C. For collagen synthesis, labelled punches were homogenized, dialysed, and then lyophilized. The lyophilized samples were subjected to bacterial collagenase digestion to release incorporated proline from collagen. After precipitation with trichloroacetic acid and centrifugation, the supernatant and pellet were counted in a liquid scintillation counter to quantitate the incorporation of [^{3}H]proline into collagen. At the end of treatment, mice were killed by CO_2 asphyxiation and dorsal skin was removed and placed in 10% buffered formalin. Paraffin-embedded sections were cut at 10 μm thickness and stained with Luna's aldehyde fuchsin for elastic fibres. The dermal repair zone is defined as the area from the epidermal-dermal junction to the top of the compressed elastotic material in the lower dermis (Kligman et al 1984; Nair et al 1991). Before the animals were killed, silicone rubber skin impressions of the dorsal area were taken. The degree of skin wrinkling was evaluated using a standardized grading system similar to that previously reported (Bissett et al 1987). Casts were observed under a stereomicroscope at 7·5 × magnification.

Repeat application skin irritation in rabbits

The hair of each rabbit was clipped closely at four dorsal sites with an electric hair clipper. Each site was a 4 cm^2. Each compound tested was applied once daily for 14 days in 200 μL of ethanol vehicle. Each day before applying drug solution, the degree of erythema, scaling and oedema was assessed visually by using the Draize (0 to 4) grading method (Draize et al 1944). On day 15, the animals were killed. The results are expressed as average daily Draize score to allow statistical comparisons of the treatments. The mean daily Draize score for each irritation parameter was calculated by summing the daily scores and dividing by 14. A strip of skin was excised from the treated sites and placed in 10% buffered formalin. These were processed, stained with H&E and evaluated for microscopic signs of inflammation (cell infiltration, oedema).

Hypervitaminosis A in CD-1 mice

Hypervitaminosis A for BMY 30123 was evaluated in CD-1 mice after intraperitoneal administration. The mice were graded daily during the treatment period using Bollag's (1974) 0 to 4 scale as illustrated in Table 1. At the end of the experiment, an animal is defined as having hypervitaminosis A syndrome if the sum of the grades from all four of the individually graded signs totals at least 3·0. Retinoids were suspended in peanut oil and injected intraperitoneally at 8 mL kg^{-1}, once daily, five days per week for two weeks. On the third day after the last treatment, mice were killed and autopsy performed. Portions of the stomach, small and large intestines, liver, dorsal skin, heart, kidney, sternum and hind limb were placed in 10% buffered formalin. Tissues were processed for histological evaluation. The development of bone fractures was assessed by histological evaluation of the long bones of the lower limbs. The grading was based on the number of fractures observed (Table 1). The sternum was also examined to provide additional histopathological evidence of hypervitaminosis A syndrome.

Table 1. Hypervitaminosis A grading system.

Sign	Degree of severity	Grade
Loss of body weight	1 g	0
	1–3 g	1
	4–6 g	2
	7–9 g	3
	> 10 g	4
Skin scaling	None	0
	Slight	1
	Moderate	2
	Severe	3
	Very severe	4
Hair loss	None	0
	Slight	1
	Moderate	2
	Severe	3
	Very severe	4
Number of bone fractures in extremities	0	0
	1	1
	2	2
	3	3
	4	4
	>4	4

Statistics

Data were analysed for significant differences by analysis of variance and Tukey's range test for multiple comparisons (Stoline 1981).

Results

Reduction of rhino mouse utriculi by BMY 30123

The skin of the rhino mouse contains numerous keratinized pilosebaceous cavities or utricles. These utricles are filled with solid impactions of horny cells resembling those in human acne comedones. Clinically active retinoids such as tretinoin and isotretinoin effectively reduce the diameter of the utricles with exfoliation of the horny material (Mezick et al 1984).

Topical application of BMY 30123 and tretinoin both caused a dose-dependent reduction in the utriculi diameters of rhino mouse skin (Fig. 2). ED30 values estimated by linear regression analysis of data compiled from several experiments for BMY 30123 and tretinoin were 0·037 and 0·015 mM, respectively. Histological observations (vertical sections) of treated skin correlated with the results of the whole mount assay and showed normalization of the epidermis and disappearance of the horn-filled utriculi. An experiment to determine the oral activity of BMY 30123 in this model showed that BMY 30123 did not have significant activity at

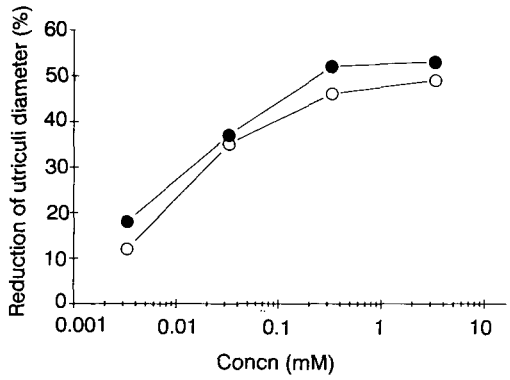

FIG. 2. Dose-dependent reduction of rhino mouse utriculi diameter by BMY 30123 (○) and tretinoin (●). Rhino mice were treated once daily for 5 days with various concentrations of test compounds in ethanol. The response was measured as described in Materials and Methods. The % reduction of utriculi diameter vs ethanol control are reported. The mean utriculi diameter values used to determine % reduction were all significantly different from the ethanol vehicle control at the $P=0.05$ level (Tukey's test). BMY 30123 was not significantly different ($P>0.05$) from tretinoin at any concentration. n = 5 mice/group.

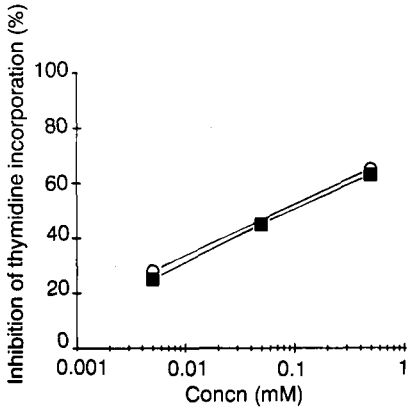

FIG. 3. Effect of test retinoids on phorbol ester (TPA)-stimulated DNA synthesis. The dorsal skin of hairless mice was treated with 0·1 mL of test retinoid in ethanol 1 h before 0·1 mL of 0·17 mM TPA was applied. Mice were killed 15 h after TPA application. [^{3}H]Thymidine incorporation into epidermal DNA was measured as described in Materials and Methods. The mean counts min^{-1} μg^{-1} DNA data used to generate the % inhibition values were all significantly different from the ethanol vehicle control at the $P=0.05$ level (Tukey's test). BMY 30123, ○; tretinoin, ■.

doses up to 50 mg kg^{-1} (data not shown). In contrast, tretinoin was active with an ED30 value of 4 mg kg^{-1}. Both compounds were dosed in peanut oil at 10 mL kg^{-1}, five days per week for two weeks.

Phorbol ester-stimulated epidermal DNA synthesis in hairless mice

Retinoids exhibit different activities depending on the initial physiological state of the skin. When applied topically to normal skin, retinoids induced epidermal hyperplasia in both man and animals (Zbinden 1975; Connor et al 1986). In comparison, retinoids appear to be effective as anti-proliferative agents in hyperproliferative epidermis. Changes in DNA synthesis, as measured by [^{3}H]thymidine incorporation, are reflective of the proliferative state of the skin. Topically applied TPA induces a large unscheduled synthesis of DNA in epidermal cells, followed by hyperplasia. It has previously been shown that pre-treatment with retinoids 1 h before TPA application results in a suppression of DNA synthesis (Gendimenico et al 1989).

BMY 30123 showed potent DNA synthesis suppression activity (Fig. 3). BMY 30123 and tretinoin blocked stimulated DNA synthesis with ED50 values of 0·15 and 0·21 μM, respectively.

Retinoid-induced hyperplasia in hairless mice

When applied to normal mouse skin, topical retinoids induce epidermal hyperproliferation (Connor et al 1986). BMY 30123, when applied topically, dose-dependently induced epidermal thickening (Table 2) as well as cellularity (data not shown). At the top dose of 3·3 mM, BMY 30123 and tretinoin increased the epidermal thickness by 255 and 221%, respectively. At the 0·33 mM dose, BMY 30123 produced a 239% increase over control while tretinoin produced a 174% increase. While both compounds stimulated epidermal hyperplasia, BMY 30123 appears to be more potent than tretinoin.

Repair of UV-induced dermal damage in hairless mice

The ability of retinoids to repair UV-induced damage to the

Table 2. Epidermal hyperplasia in hairless mouse skin induced by BMY 30123 and tretinoin.

Treatment[a] (mM)	Epidermal thickness $\mu m \pm$ s.d.	% of vehicle control
Vehicle	22·2 ± 3·4	100
BMY 30123		
3·3	56·9 ± 2·6[b]	255
0·33	53·4 ± 3·0[b]	239
0·033	37·6 ± 6·1[b]	169
Tretinoin		
3·3	49·3 ± 4·2[b]	221
0·33	38·9 ± 3·8[b]	174
0·033	33·3 ± 5·3[b]	149

[a] Flanks of male hairless mice were treated with 0·05 mL of test material in ethanol on days 0 and 1. Mice were killed on day 4. [b] Denotes that values are significantly different from vehicle control at $P<0.05$ (Tukey's test). n = 5 mice per group.

Table 3. The effect of topically applied BMY 30123 and tretinoin on the thickness of the dermal repair zone in photodamaged mouse skin.

Treatment[a]	Concn (mM)	Dermal repair zone width ($\mu m \pm$ s.d.)[b,c]
BMY 30123	3·3	89·0 ± 7·9[d,e]
	0·33	84·0 ± 8·5[d,e,f]
	0·033	69·3 ± 6·9[f,g]
Tretinoin	3·3	96·2 ± 9·3[d]
	0·33	76·9 ± 3·3[e,f,g]
	0·033	66·8 ± 9·9[f,g]
Ethanol vehicle	N/A	22·6 ± 3·3[h]
UVB/not treated	N/A	22·0 ± 4·1[h]

[a] Test materials were applied topically at 50 μL per dose, once daily, 5 days per week for 10 weeks. [b] Values having the same letter are not statistically different at $P<0.05$ (Tukey's test). [c] n = 8 mice/group.

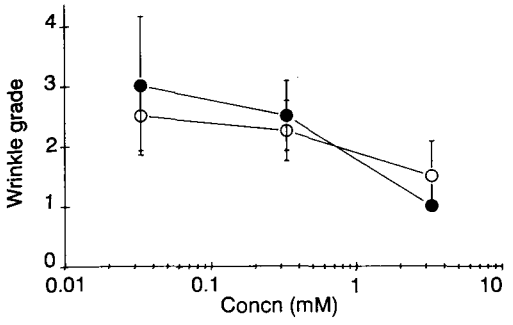

FIG. 4. The effect of tretinoin (●) and BMY 30123 (○) on UVB-induced skin wrinkling in hairless mice. Mice were exposed to UVB for 10 weeks as described in Materials and Methods. At the end of exposure, test retinoids were applied once daily, 5 days per week (0·05 mL per application) for an additional ten weeks. At the end of treatment silicone rubber casts were made of the dorsal surface of unrestrained mice. The casts were graded by visual inspection at 7·5 × magnification using a stereomicroscope. A five point grading system was employed. Grade 4 = numerous deep wrinkles; 3 = moderate number of deep and superficial wrinkles; 2 = fine wrinkling; 1 = no wrinkles; 0 = normal skin (non-UVB animals). n = 5 mice per group. The vertical bars represent s.d.

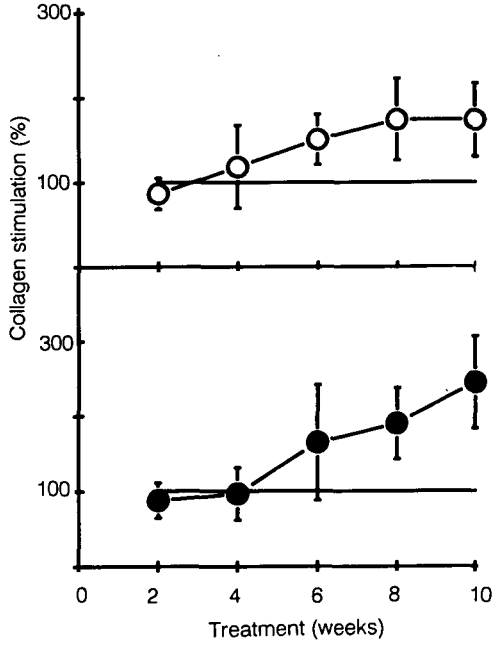

FIG. 5. The effect of tretinoin (●) and BMY 30123 (○) on collagen stimulation in the UVB-irradiated animals. Animals were irradiated with UVB for 10 weeks and treated with retinoids for an additional 10 weeks as described in Fig. 4. Significant increases in collagen synthesis were induced by both tretinoin and BMY 30123, but only after 4–6 weeks of treatment. Collagen stimulation is calculated as % collagen synthesis of the retinoid-treated animals normalized by that of the corresponding vehicle control (—). The vertical bars represent s.d.

dermal matrix in hairless mouse skin was carried out in an assay described by Kligman et al (1984) and Kligman (1986). In the absence of retinoid treatment, the UVB-treated skin sections showed extensive elastic fibre hyperplasia and disorganization of the fibres. Treatment with tretinoin results in the induction of the synthesis of a new band of extracellular matrix which appears to compress the damaged elastic fibres into a defined band. BMY 30123 and tretinoin both increased the size of the dermal repair zone (Table 3). BMY 30123 produced a fourfold increase (22·6 to 89·0 μm) in the dermal repair zone compared with the control groups. The BMY 30123 and tretinoin groups were not significantly different, at any comparable concentration, with respect to the dermal repair zone endpoint.

The effect of the test compounds on gross skin wrinkling was assessed by the evaluation of silicone skin replicas using a standardized visual grading system. The mean wrinkle grades at the end of 10 weeks of treatment are shown in Fig. 4. At the top dose (3·3 mM) both compounds effaced the UVB-induced wrinkles after 10 weeks of treatment (reduced to grade 1). The dose-response curves observed for tretinoin and BMY 30123 are identical. Both compounds effectively effaced UVB-induced wrinkles in hairless mice.

The effect of retinoids on collagen synthesis in mouse skin

Since exposure to UVB appears to damage collagen and since retinoids appear to stimulate new matrix formation in the form of a dermal repair zone, it was of interest to determine if retinoids have an effect on de-novo collagen synthesis in the skin. BMY 30123 or tretinoin was applied for 10 weeks to animals previously irradiated with UVB. Collagen synthesis was stimulated by both treatments (Fig. 5). However, there was a 4 week lag in the observed stimulation. Beginning on week 6, the observed increase in collagen synthesis of the retinoid treated animals was statistically significant relative to vehicle control. At week 10, tretinoin produced a 245% stimulation of collagen synthesis and BMY 30123 produced 173% stimulation; neither compound stimulated collagen synthesis in animals not previously exposed to UVB.

Repeat application skin irritation in rabbits

The skin irritation due to repeated topical application of BMY 30123 and tretinoin was determined in male New Zealand White rabbits. Scaling, erythema, and oedema were graded in scales ranging from 0 (no irritation) to 4 (maximal irritation). The dose-dependence of the erythema scores is shown in Fig. 6. To achieve similar irritation scores as

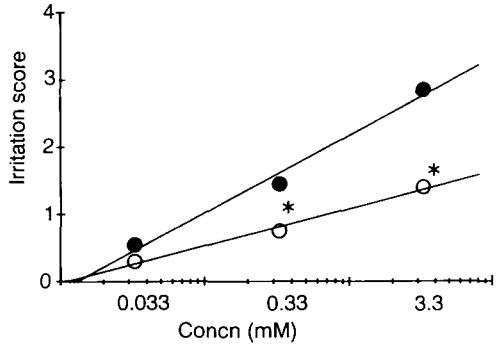

FIG. 6. Skin irritation in male New Zealand White rabbits after 14 consecutive days of application. Animals were treated as described in Materials and Methods. Erythema was assessed visually using the Draize scoring system (0 = no erythema, 4 = severe). The scores for BMY 30123 (○) were significantly different from tretinoin (●) at the $P = 0{\cdot}05$ level (Tukey's test) as noted by the asterisk (*).

observed for tretinoin, the concentration of BMY 30123 had to be increased one order of magnitude. At the concentration of 0·33 mM, tretinoin resulted in a global score similar to that of 3·3 mM BMY 30123. Similarly, 0·033 mM of tretinoin was more irritating than 0·33 mM of BMY 30123. The microscopic histopathological evaluation for irritation revealed more inflammatory cell infiltration in tretinoin-treated skin than BMY 30123-treated skin, confirming the gross findings.

The effect of topical paracetamol on skin irritation produced by tretinoin

BMY 30123 is an aromatic ester of tretinoin. Cleavage of the ester bond will give rise to two compounds; tretinoin and paracetamol. While simple ester cleavage in the skin compartment giving rise to tretinoin could explain the high potency of BMY 30123, the low skin irritation observed for this compound cannot be explained by simple ester cleavage.

An experiment was conducted to determine the effect of topical paracetamol on local skin irritation in rabbits produced by topical tretinoin. Three experimental groups were compared: 3·3 mM tretinoin; a combination of 3·3 mM tretinoinand 3·3 mM paracetamol; and 3·3 mM BMY 30123. All test agents were dissolved in ethanol.

The results are shown in Fig. 7. The combination of tretinoin and paracetamol produced the same mean daily Draize irritation scores as did tretinoin alone (erythema = 2·9). BMY 30123 produced much lower irritation (erythema = 1·2). These data suggest that simple cleavage to yield free paracetamol is not the primary factor responsible for the low irritation scores observed for BMY 30123.

Hypervitaminosis A in CD-1 mice

Hypervitaminosis A (HVA) for BMY 30123 was evaluated in CD-1 mice after intraperitoneal administration (Table 4). Bollag's scale was employed (see Table 1). BMY 30123, when dosed at 200 mg kg^{-1} showed no signs of inducing HVA. This conclusion is based on the criterion that HVA syndrome is present only when the cumulative grade is 3 or greater. In contrast, tretinoin at 100 mg kg^{-1} was so toxic that 100% of

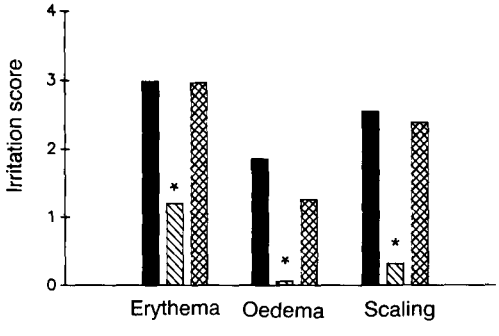

FIG. 7. The effect of combining tretinoin and paracetamol on rabbit skin irritation. Test compounds in ethanol were applied once daily, seven days per week for 14 days to male New Zealand White rabbits. For the combination of tretinoin and paracetamol, the compounds were co-dissolved to give a final concentration of 3·3 mM for each component. Tretinoin and BMY 30123 were each dosed at 3·3 mM. Erythema, oedema and scaling were assessed visually using the Draize scoring system (0 = no irritation, 4 = severe). Asterisk denotes values that are significantly different from tretinoin at $P = 0.05$ (Tukey's test). Solid, Tretinoin + paracetamol; hatched, BMY 30123; cross-hatched, tretinoin.

Table 4. Assessment of the effects of BMY 30123 and tretinoin on hypervitaminosis A (HVA) signs in CD-1 mice[a].

Treatment[a] (mg kg^{-1})	HVA Incidence	Mortality	Mean HVA[b] (grade ± s.d.)
BMY 30123			
200	0/10	0/10	0·1 ± 0·3
50	0/10	0/10	0 ± 0
20	0/10	0/10	0 ± 0
Tretinoin			
100	10/10	3/10	5·4 ± 1·5
50	4/10	0/10	2·3 ± 2·2
10	0/10	0/10	0 ± 0
Peanut oil vehicle	0/5	0/5	0 ± 0
Untreated	0/5	0/5	0 ± 0

[a] Test materials administered i.p. 8 mL kg^{-1} once daily, five days per week for two weeks. [b] Based on the 0 to 4 scale for each of the criteria described in Table 1.

the mice showed HVA symptoms and 30% of the mice died between days 7 and 10. At all doses, BMY 30123 did cause some loss of weight during days 2 through 5, but by day 10, the weight gain in these groups was comparable with the control groups. In contrast, the two higher doses of tretinoin caused weight loss during the 10 days of treatment relative to day 1. These results for tretinoin are similar to those reported by Bollag (1974). He reported that tretinoin caused HVA symptoms starting at a dose of 80 mg kg^{-1} (intraperitoneal).

Discussion

The present study demonstrates that BMY 30123, a synthetic analogue of retinoic acid, has potent topical retinoid activity in various in-vivo skin models. In addition, BMY 30123 exhibits much less skin irritation relative to tretinoin. BMY 30123 shows very little systemic retinoid activity and did not produce any evidence of hypervitaminosis A syndrome at doses twenty times greater than the no-effect dose of tretinoin.

A summary comparison of the pharmacology of BMY 30123 and tretinoin is shown in Table 5. The rhino mouse assay appears to be a reliable model for predicting retinoids that will have topical anti-acne activity in man. One of the characteristics of an acne lesion is hyperkeratinization and blockage of the hair follicles resulting in the development of comedones. Similarly, the therapeutic effects of tretinoin in acne are believed to be exerted partly through the improve-

Table 5. Pharmacological profile of BMY 30123 and tretinoin.

Test	BMY 30123 (mM)	Tretinoin (mM)
Topical rhino mouse, ED30	0·037	0·015
Oral rhino mouse	Inactive	Active
Retinoid-induced hyperplasia, ED2 × [a]	0·10	1·01
DNA synthesis inhibition, ED50	0·15	0·21
Dermal repair of photodamaged skin, ED4 × [b]	3·14	1·69

[a] ED2 × = dose required to cause a 2-fold increase in epidermal thickness vs vehicle control. Data in Table 2. [b] ED4 × = dose required to cause a 4-fold increase in dermal repair zone width relative to vehicle control. Data in Table 3.

ment of the abnormal keratinization of the follicular epithelium. In the rhino mouse assay, retinoids have been shown to affect the pattern of keratinization (Eichner et al 1986). This model has been reported to be useful for determining the anti-keratinizing activity of retinoids (Van Scott 1972; Kligman & Kligman 1979; Ashton et al 1984; Mezick et al 1984) and it appears that this model may be predictive for selecting retinoids with anti-acne activity. Available clinical data with topically used retinoids such as tretinoin and motretinide, show a correlation between their rhino mouse activity and the observed clinical effectiveness (Van Scott 1972; Lowe 1986).

In the rhino mouse assay, BMY 30123 produces a dose response curve which is nearly identical to tretinoin (BMY 30123, ED30 = 0·037 mM; tretinoin ED30 = 0·015 mM). These results suggest that BMY 30123 might have utility in the treatment of acne. BMY 30123 was devoid of oral activity in the rhino mouse assay at doses up to 50 mg kg^{-1} (data not shown). It is not clear if the lack of oral activity is due to poor oral absorption or rapid metabolism or excretion of the compound. Tretinoin has been shown to have oral activity in the rhino mouse assay (Mezick et al 1984) and was shown to have an oral ED30 of 4 mg kg^{-1} in our hands.

The induction of epidermal hyperplasia in animal skin (Connor et al 1986) by retinoids mirrors the effect of retinoids on normal human skin (Plewig & Braun-Falco 1975). BMY 30123 produces a strong response in the mouse model. The ED2× (dose required to increase epidermal thickness 2-fold) was estimated to be 0·1 mM for BMY 30123 compared with 1·01 mM for tretinoin. The increased potency observed for BMY 30123 is unexplained, but might be related to skin pharmacokinetics.

Retinoids can act differently depending on the physiological state of the skin. In hyperproliferative epidermis, such as in psoriasis, retinoids can decrease the epidermal turnover and DNA synthesis. BMY 30123 was as effective as tretinoin in suppressing phorbol ester-induced stimulation of epidermal DNA synthesis. The data suggests that BMY 30123 could be useful against disorders where epidermal cell turnover is high (e.g. psoriasis).

Chronic and excessive sun exposure is now known to produce the visible signs of aged skin. It seems clear that changes in the dermal matrix connective tissue are responsible for the visible manifestations of photo-aged skin (Smith et al 1962; Kligman 1969). Histological studies have shown that in photodamaged skin there is an increase in damaged elastic fibres, a decrease in mature collagen and a large increase in glycosaminoglycans (Kligman et al 1984; Bryce et al 1988). The mouse photodamage model reported here responds dose-dependently to topical retinoids. BMY 30123 affected all three photodamage repair endpoints. The effective dose producing a 4-fold increase (ED4×) in the dermal repair zone was 3·14 mM for BMY 30123 and 1·69 mM for tretinoin. Both compounds effectively effaced the gross skin wrinkling induced by UVB. The mechanism of wrinkle effacement is not clear at this time. One possible mechanism might be the synthesis of new collagen which changes the biomechanical properties of the dermis. Both tretinoin and BMY 30123 stimulated de-novo collagen synthesis in animals previously exposed to UVB. It is of interest to note that there was a lag time observed for the stimulation of new collagen synthesis. Detectable levels of stimulation over control were not apparent until 4–6 weeks after initiating treatment with the retinoid. This same lag was also observed in the improvement of wrinkles suggesting that new collagen synthesis may have a role in the retinoid-induced wrinkle effacement. Further work is needed to completely understand the mechanisms of retinoid-induced acceleration of repair in UVB damaged skin.

The topical skin irritation data demonstrate that BMY 30123 produces lower local skin irritation than tretinoin. The dose-response studies on rabbit irritation show that BMY 30123 is at most one-tenth as potent as a skin irritant than tretinoin. Since the ED30 values for BMY 30123 and tretinoin are not significantly different, the comparison of the therapeutic index (irritation/activity) between the two compounds depends mainly on irritation. Therefore, the therapeutic index of BMY 30123 is 10 times higher than that calculated for tretinoin. This gives BMY 30123 a major topical safety advantage with respect to skin irritation.

BMY 30123 also showed very little systemic retinoid toxicity. After intraperitoneal injection, BMY 30123 did not produce signs of hypervitaminosis A syndrome at doses 20 times higher than the no-effect dose of tretinoin. Since retinoid efficacy was not observed after oral administration, this lack of systemic toxicity may be less meaningful. However, the lack of systemic activity after topical application could be significant in the long term use of a topical retinoid applied to large surface areas of permeable skin. Such use would be expected in the treatment of photodamaged skin. BMY 30123 could have an additional safety factor in such cases where long term exposure and greater body surface application results in higher systemic drug load.

BMY 30123 is the paracetamol ester of tretinoin. It is theoretically possible that this ester would be cleaved in the skin to give the parent compound tretinoin and free paracetamol. However, this does not appear to be the case, since physical mixtures of tretinoin and paracetamol, which represent the 100% hydrolysis product, have different properties to BMY 30123. The skin irritation produced by the mixture is as high as tretinoin, which is much higher than intact BMY 30123. Preliminary skin metabolism work (data not shown) suggests that BMY 30123 is not simply cleaved in the skin to give tretinoin.

The pharmacological properties of BMY 30123 clearly show that separation of retinoid efficacy from skin irritation is possible. The molecular mechanism by which this is achieved is not clear. However, the pharmacological/toxicological profile obtained with BMY 30123 suggests that it will have clinical utility in the treatment of dermatological disorders without some of the side effects associated with topical retinoid therapy.

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