J. Pharm. Pharmacol. 1995, 47: 626–631
Received October 10, 1994
Accepted January 13, 1995

In-vivo Activity of Retinoid Esters in Skin is Related to In-vitro Hydrolysis Rate

SIMON CHEN, INGER M. DARLING, KUO-LONG YU*, JOHN E. STARRETT JR.*, MUZAMMIL M. MANSURI*, GARY WHITING AND KENNETH M. TRAMPOSCH

*Department of Biochemical Pharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, 100 Forest Avenue, Buffalo, NY 14213, and *Central Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

Abstract

BMS-181163 (4-acetamidophenyl retinoate, previously reported as BMY-30123), the acetamidophenyl ester of all-*trans*-retinoic acid (tRA), is topically active in various retinoid-sensitive animal models, but was recently shown to be ineffective for the treatment of acne in patients. To determine whether BMS-181163 functions as a prodrug of tRA in mice but not in man, the relative rates of ester hydrolysis in mouse and human skin homogenates were determined.

In-vitro hydrolysis assays showed that BMS-181163 was substantially hydrolysed in mouse skin homogenates and minimally in human skin preparations. In addition, a series of phenyl esters of tRA and several known active synthetic retinoids (Ch-80: (E)-4-[3-oxo-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid; CD-271: 6-[3-(1-adamantyl)-4-methyoxyphenyl]-2-naphthoic acid; and TTNPB: (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid) was prepared and hydrolysis rates and in-vivo (rhino mouse utriculi reduction) activities were compared. The hydrolysis rates of the six test retinoid phenyl esters, ranging from 0·06 to 2·0 h^{-1}, were found to correlate with the in-vivo activity. Those esters (BMS-181163 and acetamidophenyl esters of Ch-80 and TTNPB) with a higher hydrolysis rate exhibited in-vivo activity only slightly lower than their parent free acid retinoids. In contrast, the three phenyl esters with a hydrolysis rate less than 0·3 h^{-1} were inactive in-vivo.

Data from both approaches suggest that the breakdown of the phenyl ester linkage in this series is essential for the respective in-vivo activity and that the active phenyl esters of retinoids function as prodrugs.

Topical retinoids demonstrate beneficial effects on the clinical conditions of acne (Kligman et al 1969), psoriasis (Orfanos et al 1979), and skin photodamage (Kligman et al 1986; Leyden et al 1989). Skin irritation, however, is an unwanted side effect (Gilchrest 1991) associated with topical retinoid treatment. In this regard, attempts have been made to identify new retinoids with an improved therapeutic index. Recently, a retinoic acid ester, BMS-181163, was shown to be topically active in a number of retinoid-sensitive skin models including the rhino mouse utriculi reduction assay, the hairless mouse photoaging model, the hairless mouse epidermal hyperplasia model and in the suppression of DNA synthesis in mouse skin stimulated with phorbol ester (Tramposch et al 1992). In this same study, BMS-181163 was reported to exhibit very low skin irritation in rabbits, thus making it an ideal candidate for use in various dermatological disorders. However, clinical evaluation of a 0·3% cream formulation showed that BMS-181163 was not significantly better than vehicle in treatment of acne vulgaris (Warren Epinette, personal communication). Assuming that BMS-181163 is a prodrug of all-*trans*-retinoic acid (tRA), this discrepancy might be related to the species differences in the rate of ester hydrolysis.

Esterases are present in both mouse (Ghosh & Mitra 1990) and human (Rawlins et al 1979; Bonina et al 1991) skin, and esters have been designed as prodrugs to facilitate delivery through rat and human skin (Seki et al 1990; Bonina et al 1991). Therefore, the relative hydrolysis rates of BMS-181163 were investigated using supernatants of hairless mouse and human skin homogenates.

Correspondence: S. Chen, Department of Biochemical Pharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, 100 Forest Avenue, Buffalo NY 14213, USA.

Materials and Methods

Retinoids

All-trans retinoic acid (tRA) was purchased from Eastman Kodak Company (Rochester, NY). BMS-181163 (previously reported as BMY-30123 in Tramposch et al 1992), ester **5** [2-(benzothiazolyl)phenyl retinoate] and ester **6** [2-*n*-propylphenyl retinoate] were synthesized by Eastman Kodak Company (Rochester, NY). All other retinoids were synthesized in Central Chemistry, Bristol-Myers Squibb, Wallingford, CT.

Animals

Six- to nine-week-old female hairless rhino mice (hr^{rh}/hr^{rh}) were produced in the Bristol-Myers Squibb colony. Eight- to ten-week-old albino hairless mice (Skh:HR1) were purchased from Charles River Laboratories (Wilmington, MA). Animals were acclimatized for one week before the study. Animals were kept on a 12 h light : dark cycle and had free access to food (Agway Inc., Syracuse, NY) and water,

according to NIH guidelines. All procedures involving retinoids were carried out under yellow light to minimize photoisomerization.

Human skin

Fresh human skin samples were obtained from the National Cancer Institute, Cooperative Human Tissue Network (Columbus, OH). All samples were female breast skin taken from surgical patients and were determined to be normal skin in pathology reports. Skin was received on ice in saline on the day following surgery.

Rhino mouse utriculi reduction assay

The rhino mouse utriculi assay was modified from Ashton et al (1984) as previously reported (Tramposch et al 1992). In brief, test retinoids in ethanol vehicle (50 μL) were applied to the dorsal area (approx. 1·5×3 cm^2) of rhino mice once daily for 5 days (Monday to Friday). For various retinoids, a dose response was obtained with concentrations ranging from 0·00033 to 3·3 mM (equivalent of daily application of 16·7 pmol to 0·167 μmol). The animals were killed on the following Monday by CO_2 inhalation. A 7/8″ full thickness punch was taken from the central dorsal area of each animal. The epidermis of the biopsy was removed from the dermis after incubation in 0·5% acetic acid overnight at 4°C. The separated epidermis was then fixed in formalin, dehydrated with ethanol, and cleared in xylene. To determine the utriculi diameter, each epidermis sheet was placed on a glass slide in xylene. For each specimen, the diameter of 49 utricules was measured with an image analysis system (IBM PC, Image Measure program and Olympus microscope with video camera). Percentage utriculi reduction was calculated as:

$$\left(1 - \frac{\text{utriculi diameter in the test group}}{\text{utriculi diameter in the ethanol control group}}\right) \times 100$$

In-vitro skin homogenate preparation

Skin homogenates were prepared from dorsal skin of naive rhino and hairless mice or human skin. Mouse skin was removed after death by CO_2 inhalation. Total skin was weighed, chopped into small pieces and frozen in liquid nitrogen. The frozen skin samples were pulverized in a freezer/mill (Model 6700, SPEX Industries, Edison, NJ) and diluted with 10 vol (w/v) 0·05 M phosphate buffer, pH 7·4. This solution was homogenized further using a tissue homogenizer (Kinematica GmbH, Switzerland, two 10-s pulses) to suspend all the tissue. Samples were centrifuged at 100 *g*, at 4°C for 20 min. Protein concentration of the 1000 *g* supernatant was determined using a Bradford dye-binding assay with gamma-globulin as the standard protein (Bio-Rad Laboratories, Hercules, CA). The homogenates were adjusted to 2 mg protein mL^{-1} for the metabolism studies. Human skin was cleaned using phosphate-buffered saline to remove residual fat from the sample. This was then treated similarly to the mouse skin.

Skin metabolism studies

Homogenate supernatant was incubated at 37°C for 10 min before initiation of the study. At time zero a small aliquot of the compound was added to the supernatant to achieve approximately 2·5 μM concentration. The compounds were dissolved in ethanol and the final concentration of ethanol in the incubations was 1% or less. After vortexing, a small sample was removed and immediately frozen in a dry-ice/methanol bath to represent the initial concentration of compound. The rest of the sample was incubated at 37°C in a shaking water-bath and aliquots were removed at appropriate times and flash frozen. The samples were stored at −80°C until analysis by HPLC.

HPLC analysis

The HPLC system consisted of two Model 501 pumps, an Automated Gradient Controller, a Model 712 WISP, a Model 484 Tunable Absorbance Detector, a Model 746 Data Module and a Nova-Pak C18, 3·9×150 mm HPLC column with a Nova-Pak C18 Guard-Pak precolumn.

All samples were analysed using reverse phase HPLC. Retinoid samples were prepared for analysis by precipitating proteins with a 2:1 dilution with acetonitrile. The mobile phase consisted of a mixture of methanol and 0·05 M ammonium acetate, pH 4·2 with a flow rate of 1·0 mL min^{-1}. The percent composition varied for each compound analysed to achieve separation of the ester from the free acid. The effluent was monitored at the UV maxima for each of the compounds. Retinyl acetate was used as an internal standard for the analysis of BMS-181163. Standards and unknown samples were alternated throughout the assay period to verify appropriate injection volumes for those compounds that were analysed without internal standards.

Betamethasone, betamethasone-17-valerate and betamethasone-21-valerate were analysed using a gradient elution. The initial mobile phase was methanol : water (58 : 42) and changed in a linear fashion to 72 : 28 composition at 5·5 min with a flow rate of 1·0 mL min^{-1}. The retention times for betamethasone, betamethasone-17-valerate and betamethasone-21-valerate were 5·4, 15·3 and 16·3 min, respectively. Standards were unavailable for betamethasone-21-valerate; therefore, measurements of the formation of betamethasone-21-valerate were based on the betamethasone-17-valerate standards assuming a similar extinction coefficient.

Results

BMS-181163 hydrolysis in hairless mouse and human skin

The breakdown of BMS-181163 in mouse and human skin supernatant incubations is shown in Fig. 1. Substantial loss of compound was observed in the hairless mouse supernatant whereas no appreciable breakdown was detected in the human skin preparation. The breakdown observed was due to enzymatic metabolism (specifically esterases) as either heat denaturation or the presence of physostigmine (1 mM) substantially reduced the loss of compound. All-*trans*-retinoic acid was not observed in these incubations due to rapid breakdown (data not shown). Upon incubation of tRA alone in these homogenates, the rate of loss of tRA was greater than that of BMS-181163 (only 4% intact tRA remaining after 1 h).

To verify that the lack of hydrolysis of BMS-181163 in the human skin preparation did not result from the absence of esterase activity, the conversion of betamethasone-17-vale-

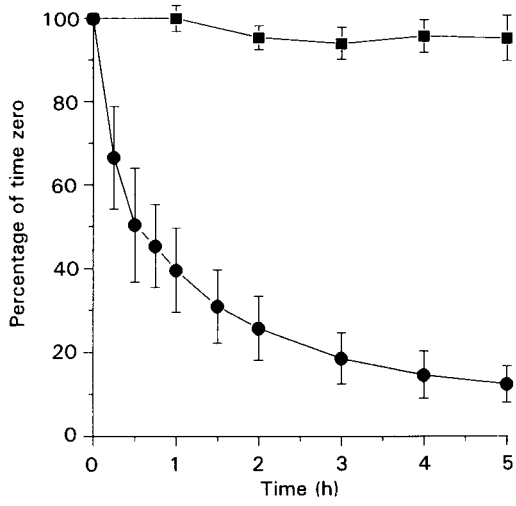

FIG. 1. Breakdown of BMS-181163 in hairless mouse and human skin homogenates. Data is presented as the mean ± s.d. (n = 3). ● Mouse-skin, ■ human skin.

rate into betamethasone in the skin supernatant preparations was investigated (Rawlins et al 1979). Betamethasone-17-valerate isomerizes to betamethasone-21-valerate which is then acted upon by esterases to release betamethasone into the supernatant (Yip & Li Wan Po 1979). Non-quantifiable concentrations of betamethasone-21-valerate were observed in the incubation mixtures, probably due to betamethasone being formed immediately upon isomerization of the 17-valerate to the 21-valerate form. Fig. 2 shows that betamethasone was converted from betamethasone-17-valerate at comparable rates in human and mouse skin supernatants, thereby confirming the presence of active esterase enzymes in both mouse and human homogenate supernatants.

Rhino mouse utriculi reduction activity of phenyl esters

To further expand the in-vitro/in-vivo comparison, five other phenyl esters were drived from BMS-181163 by changing either the retinoid (esters **2–4**) or the ester (esters **5–6**) part of the molecule. In esters **2–4** (Fig. 3; Table 1), tRA was substituted with other known synthetic retinoids: Ch-80 (Kagechika et al 1989), TTNPB (Chatelus et al 1989) and CD-271 (Bernerd et al 1991). The utriculi reduction activities of the parent retinoids and the corresponding acetamidophenyl esters are compared in Figs 4A–D. Acetamidophenyl ester derivation had slight effect on the in-vivo activity of tRA, Ch-80 and TTNPB. Although the parent retinoids were more potent, the acetamidophenyl ester derivatives of these three retinoids (BMS-181163, esters **2** and **3**) remained highly active. In all three cases, acetamidophenyl esterification reduced the activity of parent retinoids by one order of magnitude or less. In contrast, the acetamidophenyl ester analogue of CD-271 (ester **4**) was devoid of in-vivo activity. In addition to the acetamidophenyl esters, esters **5** and **6** were derived from BMS-181163 by replacing the acetamidophenyl ester with either benzothiazolylphenyl or propylphenyl ester (Table 1). These two phenyl esters of tRA were both inactive in the rhino mouse assay (Figs 4E–F). For esters **4–6**, esterification clearly diminishes the in-vivo activity of their parent retinoids.

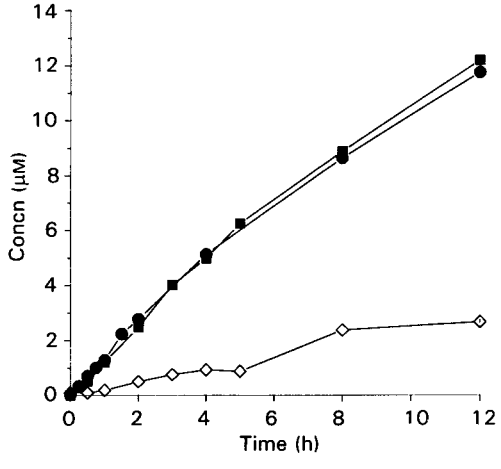

FIG. 2. Presence of esterase activity in human and hairless mouse skin supernatants. Formation of betamethasone from betamethasone-17-valerate was measured in mouse and human skin homogenates. ● Mouse skin, ■ human skin, ◇ incubation buffer alone.

Hydrolysis of retinoid phenyl esters in rhino mouse skin homogenate supernatants

The in-vitro hydrolysis rates of retinoid esters in the rhino mouse supernatants are shown in Table 1 and Fig. 5. BMS-181163, esters **2** and **3** were quickly broken down in-vitro with hydrolysis rates ranging from 0·5 to 2 h^{-1} and half-lives from 0·35 to 13 h. In contrast, esters **4–6** were more stable, with hydrolysis rates lower than 0·3 h^{-1} and half-lives greater than 2·5 h. That ester hydrolysis was the cause of compound breakdown is confirmed in Fig. 6, in which the disappearance of the acetamidophenyl ester analogues corresponds to the appearance of the parent free-acid retinoids. Comparison between Table 1 and Fig. 4 indicates that the in-vivo activity of retinoid esters correlates with their in-vitro hydrolysis rates. Esters with a hydrolysis rate lower than 0·3 h^{-1} were not active in-vivo.

Discussion

Retinoic acid displays a wide spectrum of biological activity in various tissues (Shapiro 1986; Uitto & Olsen 1989). This pleiotropic effect on one hand renders retinoic acid useful in the treatment of several clinical conditions, and on the other hand generates undesirable side-effects. In an attempt to identify a retinoid with an improved therapeutic index, several acetamidophenyl derivatives of active parent retinoids were synthesized and evaluated. Of these compounds, BMS-181163, the acetamidophenyl derivative of all-*trans* retinoic acid, demonstrated efficacy in numerous dermatological animal models and a surprisingly low irritation profile in rabbits (Tramposch et al 1992). However, in clinical trials, topical BMS-181163 was found to be ineffective for the treatment of acne vulgaris.

The mechanism for the lack of effect of BMS-181163 in man and substantial efficacy in animals could be related to

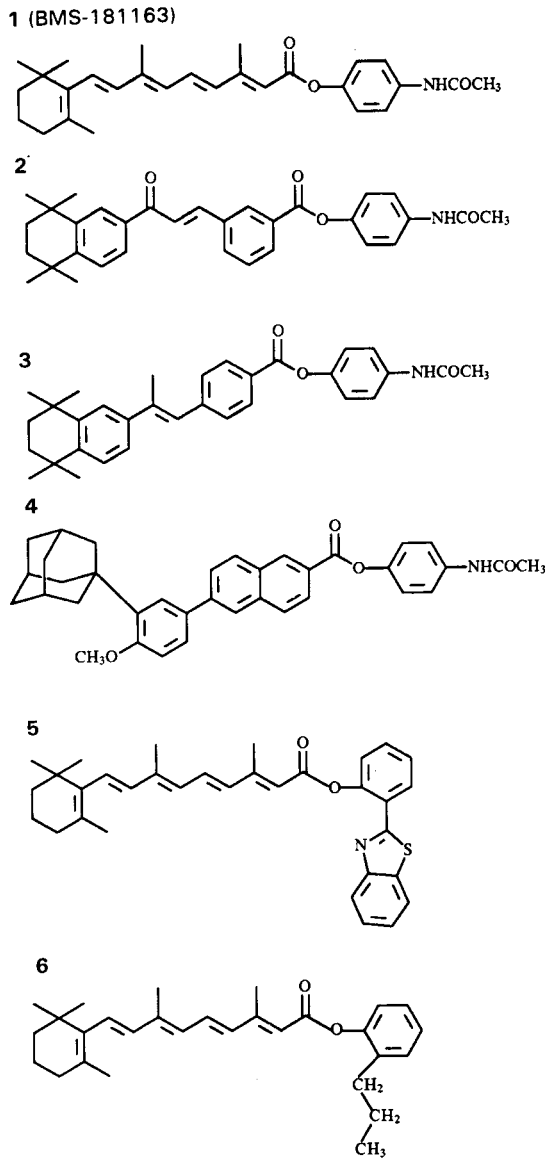

FIG. 3. Structures of ester compounds **1**–**6**.

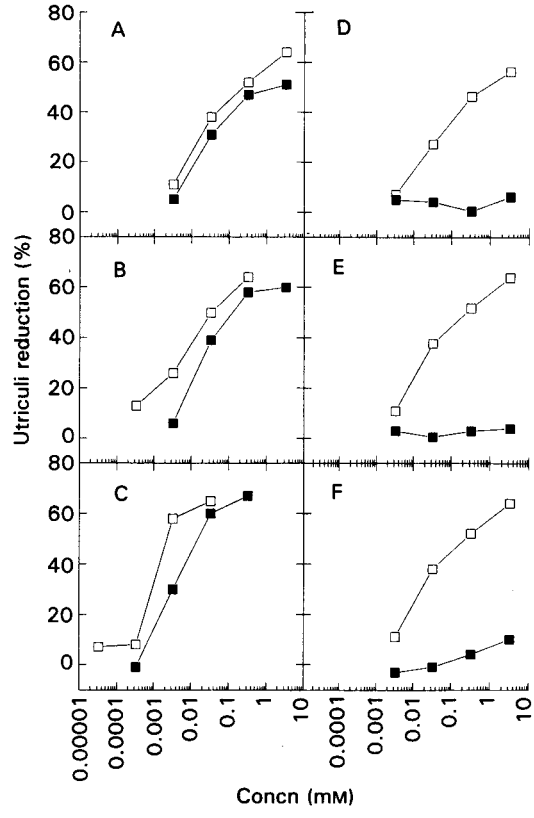

FIG. 4. Utriculi reduction activity of the parent retinoids and corresponding acetamidophenyl ester derivatives. □ Parent retinoids (tRA (A), Ch-80 (B), TTNPB (C), CD-271 (D), tRA (E), tRA (F)), ■, corresponding acetamidophenyl esters (BMS-181163 (A); ester **2** (B); ester **3** (C); ester **4** (D); ester **5** (E); ester **6** (F)).

either absorption differences or differential ester hydrolysis between species. Our studies comparing the topical absorption of BMS-181163 and Retin-A cream formulations in rats revealed no substantial difference in the systemic absorption (data not shown). Although this was studied in rats and not man, it suggests that availability of the compound from the cream formulation may not be the cause of the lack of effect.

Another possibility is that BMS-181163 is a prodrug of tRA and is hydrolysed differentially in human and mouse skin. In this regard, it is interesting to point out that although esterase activity is present in mouse (Ghosh & Mitra 1990) and man (Rawlins et al 1979; Bonina et al 1991) skin, the enzyme activity or specificity in skin may vary considerably even between different strains of mice (Ghosh & Mitra 1990). Furthermore, Rao & Kaveeshwar (1992) showed that plasma carboxylesterase activity was higher in mouse than in man. Hydrolysis of BMS-181163, therefore,

Table 1. Structures, hydrolysis rates and half-lives of phenyl esters.

	Parent retinoid	Ester	k (h^{-1})	$t_{\frac{1}{2}}$ (h)
1 (BMS-181163)	tRA	Acetamidophenyl	1·01 ± 0·13	0·70 ± 0·10
2	Ch-80	Acetamidophenyl	1·99 ± 0·10	0·35 ± 0·02
3	TTNPB	Acetamidophenyl	0·52 ± 0·01	0·33 ± 0·03
4	CD-271	Acetamidophenyl	0·06 ± 0·03	14·2 ± 8·6
5	tRA	Benzothiazolylphenyl	0·21 ± 0·06	3·49 ± 1·12
6	tRA	Propylphenyl	0·26 ± 0·02	2·66 ± 0·24

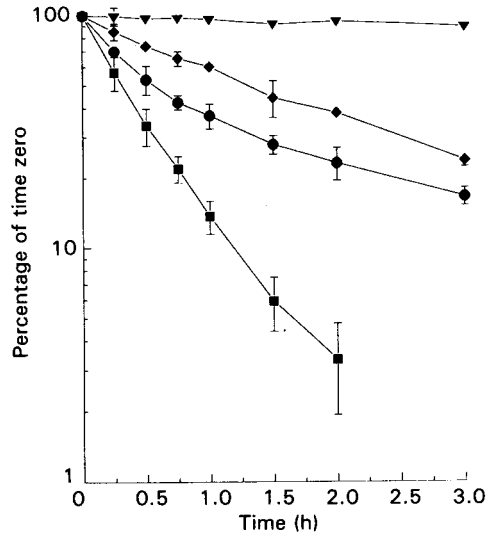

FIG. 5. Ester hydrolysis of various retinoid esters in rhino mouse skin homogenates. Data is represented as the mean ± s.d. (n = 3). ●, BMS-181163; ■, ester **2**; ◆, ester **3**; ▼, ester **4**.

was examined in human and hairless mouse skin supernatants (Fig. 1). BMS-181163 was readily hydrolysed in mouse skin preparation, whereas in human skin, breakdown of BMS-181163 hardly occurred. Approximately 90% BMS-181163 still remained intact after 5 h of incubation with the human skin supernatant. That this difference was not due to a lack or loss of esterase activity in the human skin preparation was demonstrated by its ability to convert betamethasone-17-valerate into betamethasone. Since the formation of betamethasone was comparable in the two preparations (Fig. 2), the differential hydrolysis of BMS-181163 most likely resulted from the different esterase specificity in human and mouse skin.

These results establish that BMS-181163 may function as a prodrug in mouse but not in human skin. The acetamidophenyl group needs to be removed to reveal the free acid for biological activity. Interestingly, the importance of a free polar end-group in retinoids is suggested by some recent findings. Retinoids are believed to exercise their diverse biological functions by binding to the nuclear retinoic acid receptors (RARs). Three RARs (α, β, and γ) have been reported so far (for review, see Mangelsdorf et al 1994). In-vitro binding of numerous synthetic retinoids to RARs revealed that the free terminal carboxylic acid was essential for high receptor binding affinity (Apfel et al 1991), suggesting that the interaction of the polar end-group in retinoids with the receptors is very important. Since BMS-181163 cannot be converted into its free acid metabolite (tRA) in human skin, presumably it cannot initiate the nuclear receptor cascade.

To further expand the comparison between the hydrolysis rate of retinoid esters and their in-vivo potency, five other phenyl esters were synthesized (Table 1). Of the six esters, phenyl esters **1–3** were highly active in the rhino mouse utriculi assay (Fig. 3). Esters **4–6**, on the contrary, were inactive in-vivo. The three active esters have high hydrolysis rates ($> 0{\cdot}5\,h^{-1}$) whereas the three inactive esters have low hydrolysis rates ($< 0{\cdot}3\,h^{-1}$) in-vitro. It thus appears that when the hydrolysis rate is lower than $0{\cdot}3\,h^{-1}$, the turnover of the esters into their corresponding active parent retinoids may not be rapid enough to trigger detectable rhino mouse activity.

The data in Table 1 and Fig. 4 also indicate that the skin esterase system is capable of recognizing structural differences besides the ester linkage. In the acetamidophenyl ester group (esters **1–4**), for example, only phenyl ester **4** showed

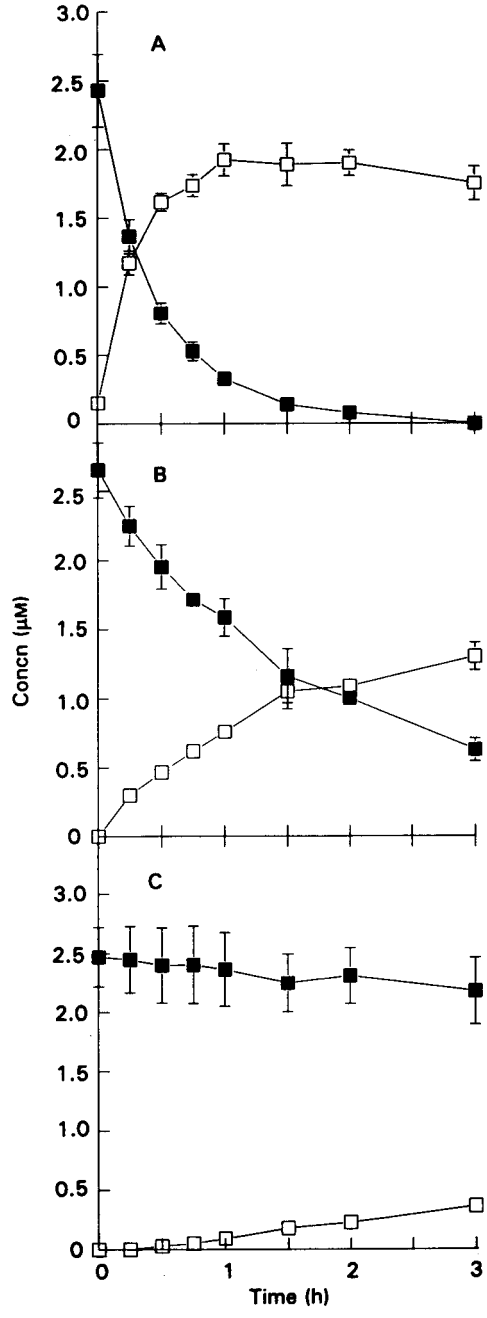

FIG. 6. Formation of free acid retinoids from respective esters. Data represents the mean ± s.d. (n = 3). ■, Acetamidophenyl esters of retinoids (ester **2** (A); ester **3** (B); ester **4** (C)); □, corresponding parent retinoids (CH-80 (A); TTNPB (B); CD-271 (C)).

a relatively slow hydrolysis rate in mouse skin supernatant. The other three acetamidophenyl esters were readily metabolized. Similarly, when comparing the three derivatives of tRA (BMS-181163, esters **5** and **6**), BMS-181163 was easily hydrolysed, but not phenyl ester **5** or **6**. These observations, along with the finding that BMS-181163 was hydrolysed in mouse but not in human skin, suggest that the skin esterase systems can have high substrate specificity.

This study demonstrates that mouse skin has the capability to rapidly hydrolyse certain retinoid phenyl esters and that these esters probably serve as prodrugs in mouse skin. The fact that human skin does not readily hydrolyse BMS-181163 suggests that the lack of clinical activity of this compound may be related to this finding.

References

Apfel, C., Crettaz, M., Siegenthaler, G., Hunziker, W. (1991) Synthetic retinoids: differential binding to retinoic acid receptors. In: Saurat, J.-H. (ed.) Retinoids 10 Years On. Karger, Basel, pp 110–120

Ashton, R. E., Connor, M. J., Lowe, N. J. (1984) Histologic changes in the skin of the rhino mouse ($hr^{rh}hr^{rh}$) induced by retinoids. J. Invest. Dermatol. 82: 632–635

Bernerd, F., Ortonne, J.-P., Bouclier, M., Chatelus, A., Hensby, C. (1991) The rhino mouse model: the effect of topically applied all-*trans* retinoic acid and CD271 on the fine structure of the epidermis and utricle wall of pseudocomedones. Arch. Dermatol. Res. 283: 100–107

Bonina, F. P., Montenegro, L., De Capraris, P., Bousquet, E., Tirendi, S. (1991) 1-Alkylazacycloalkan-2-one esters as prodrugs of indomethacin for improved delivery through human skin. Int. J. Pharm. 77: 21–29

Chatelus, A., Caron, J. C., Shroot, B., Eustache, J., Hensby, C. N. (1989) Structure-activity relationships between different retinoids using the topical rhino mouse comedolytic model. In: Reichert, U., Shroot, B. (eds) Pharmacology of Retinoids in the Skin. Vol. 3 Karger, Basel, pp 144–148

Ghosh, M. K., Mitra, A. K. (1990) Carboxylic ester hydrolase activity in hairless and athymic nude mouse skin. Pharm. Rev. 7: 251–255

Gilchrest, B. A. (1991) Retinoid pharmacology and skin. In: Mukhtar, H. (ed.) Pharmacology of the Skin. CRC Press, London, pp 167–181

Kagechika, H., Kawachi, E., Hashimoto, Y., Shudo, K. (1989) Retinobenzoic acids. 2. Structure-activity relationships of chalcone-4-carboxylic acids and flavone-4′-carboxylic acids. J. Med. Chem. 32: 834–840

Kligman, A. M., Fulton, J. E., Plewig, G. (1969) Topical vitamin A acid in acne vulgaris. Arch. Dermatol. 99: 469–476

Kligman, A. M., Grove, G.L., Hirose, R., Leyden, J. J. (1986) Topical tretinoin for photoaged skin. J. Am. Acad. Dermatol. 15: 836–859

Leyden, J. J., Grove, G., Grove, M. J., Thorne, E. G., Lufrano, L. (1989) Treatment of photodamaged facial skin with topical tretinoin. J. Am. Acad. Dermatol. 21: 638–644

Mangelsdorf, D. J., Umesono, K., Evans, R. M. (1994) The retinoid receptors. In: Sporn, M. B., Roberts, A. B., Goodman, D. S. (eds) The Retinoids: Biology, Chemistry, and Medicine, 2nd edn. Raven Press, New York, pp 319–349

Orfanos, C. E., Mahrle, G., Goerz, G., Happle, R., Hofbauer, M., Landes, E., Schimpf, A. (1979) Laboratory investigations in patients with generalized psoriasis under oral retinoid treatment: a multicenter study of computerized data. Dermatologica 159: 62–70

Rawlins, M. D., Shaw, V., Shuster, S. (1979) The in vitro metabolism of betamethasone-17-valerate by human skin. Br. J. Pharmacol. 66: 411P

Rao, S. S., Kaveeshwar, U. (1992) Difference in the inhibition of plasma carboxylesterase activity by metoclopramide in humans and laboratory animals. Pharmazie 47: 391

Seki, T., Kawaguchi, T., Juni, K. (1990) Enhanced delivery of Zidovudine through rat and human skin via ester prodrugs. Pharm. Res. 7: 948–952

Shapiro, S. S. (1986) Retinoids and epithelial differentiation. In: Sherman, M. I. (ed.) Retinoids and Cell Differentiation. CRC Press, New Jersey, pp 29–60

Tramposch, K. M., Nair, X., Gendimenico, G. J., Tetrault, G. B., Chen, S., Kiss, I., Whiting, G., Bonney, R. J. (1992) The pharmacology of a novel topical retinoid, BMY-30123: comparison with tretinoin. J. Pharm. Pharmacol. 44: 379–386

Uitto, J., Olsen, D. R. (1989) Retinoid modulation of cutaneous extracellular matrix gene expression. In: Reichert, U., Shroot, B. (eds) Pharmacology of Retinoids in the Skin. Vol. 3. Karger, Basel, pp 37–44

Yip, Y. W., Li Wan Po, A. (1979) The stability of betamethasone-17-valerate in semi-solid bases. J. Pharm. Pharmacol. 31: 400–402