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Effects of Retinoids on Type IV Collagenolytic Activity in Melanoma Cells

AARNE OIKARINEN and TUULA SALO

Departments of Dermatology and Medical Biochemistry, University of Oulu, Oulu, Finland

Oikarinen A, Salo T. Effects of retinoids on type IV collagenolytic activity in melanoma cells. Acta Derm Venereol (Stockh) 1986; 66: 346–348.

The effects of retinol, all-trans-retinoic acid, isotretinoin and etretinate on the activity of basement membrane collagen degrading enzyme was studied in melanoma cells. The results indicated that retinoids at concentrations of up to 10^{-6} M did not significantly affect type IV collagenolytic activity in these cells in vitro. Since type IV collagenolytic enzyme may be involved in the metastatic potential of tumour cells, it appears that retinoids do not affect the metastatic potential of melanoma cells by affecting type IV collagenolytic activity. *Key word: Type IV collagenase.* (Received February 13, 1986.)

A. Oikarinen, Department of Dermatology, University of Oulu, SF-90220 Oulu, Finland.

Retinoids are used extensively for the treatment of various dermatological diseases (1). New derivatives of vitamin A have also been suggested to have anti-tumour effects and these retinoids would be particularly useful for the treatment of epithelial tumours (2, 3). There are some studies indicating that retinoids decrease the number of certain types of epitheliomas such as basal cell carcinomas, or premalignant lesions such as solar keratosis. Retinoids have also been shown to decrease the proliferation rate of normal and malignant cells (3). For tumour growth, the ability of tumours to invade and penetrate basement membranes is essential (4). It has been shown that malignant tumours produce specific proteolytic enzymes which can degrade basement membrane collagen (type IV) (4–6). In some studies the production of type IV collagenase correlated well with the metastatic potential of malignant cells (6). In the present study the effects of various retinoids on type IV collagenolytic activity were studied in human melanoma cells (A 2058).

MATERIAL AND METHODS

Retinoids

The unlabelled retinoids, all-trans-retinoic acid and retinol were purchased from the Sigma Chemical Co; isotretinoin (13-Cis-retinoic acid), etretinate (RO-10-9359), and the free acid of etretinate (RO-10-1670) were obtained as a gift from La Roche, Basel. The radioactive retinoid 11-^{3}H all-trans-retinoic acid (specific activity 2.24 Ci/mmol) was obtained from the Chemoprevention Program, National Cancer Institute, National Institutes of Health.

The assay of type IV collagenase

For the enzyme activity determinations melanoma cell cultures were washed with phosphate-buffered saline to remove traces of serum, and medium was replaced with serum-free DMEM containing 0.25% bovine serum albumin, 0.1% transferrin, retinoid or ethanol. After an incubation time of up to 48 h, the cells were counted and the cell culture medium was collected after centrifugation for 10 min at 600 g to remove cells and debris, and the supernatant was stored frozen.

The type IV collagenolytic activity was assayed in cell culture medium proteins as described previously (5) by using soluble [^{3}H]proline-labelled type IV procollagen as a substrate (7). Briefly, enzyme samples were activated with trypsin (10 μg/ml), followed by the addition of soyabean trypsin inhibitor (40 μg/ml), N-ethylmaleimide (4 mM), aprotinin (1 000 KIU/ml) and substrate 3000 cpm). the reaction was carried out for 18 h at 35°C and was terminated by adding 20 μl of bovine serum albumin (1 mg/ml) and 100 μl of a solution containing 10% trichloroacetic acid and 5% tannic acid. The mixture was incubated on ice and the undigested material was precipitated and removed by centrifugation at 5000 g for 15 min. Radioactivity in the supernatant was measured in a scintillation counter.

The assay of cellular retinoic acid binding protein (CRABP)

For cytosol binding assay, frozen melanoma cell pellets were hemogenized on ice with Teflon-glass homogenizer (20 strokes) in 1.0 ml of 20 mM Tris-Cl, pH 7.5, containing 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10% glycerol. The homogenates were centrifuged at 100000 g for 60 min at 4°C, and the supernatants were used for receptor binding assay as described earlier (8) and for protein determination (9).

RESULTS AND DISCUSSION

In melanoma cells, various retinoids at a concentration of 10^{-6} M did not markedly affect type IV collagenolytic activity (Table I). Trypsin activation of type IV collagenase did not affect the results, indicating that retinoids do not affect on the ratio of latent type IV collagenase to active form of the enzyme. The variation of pre-incubation time with retinoids (24–48 hours) did not affect the results. Lower concentrations of retinoids (up to 10^{-9} M) also had no effect on enzyme activity. Our preliminary studies have indicated that retinoids do not affect type IV collagenolytic activity in other models tested, e.g. mouse tumour (unpublished results).

Table I. *Effects of various retinoids on type IV collagenolytic activity in melanoma cells*[a]

Retinoid	(M)	Trypsin −		Trypsin +		Trypsin activatable/ active enzyme
		CPM	%	CPM	%	
Control	–	228	100	404	100	1.72
Retinol	10^{-6}	229	100	381	94	1.66
All-trans-retinoic acid	10^{-6}	275	121	n.d.		–
Isotretinoin	10^{-6}	246	107	418	103	1.70
Etretinate	10^{-6}	278	122	440	109	1.58
Free acid of etretinate	10^{-6}	203	89	438	108	2.12

[a] The melanoma cells were preincubated for 48 hours with retinoids before collecting the media for enzyme assays. The enzyme was assayed before and after trypsin (3 mg/ml) activation.

Since most tissues contain cellular retinoic acid binding protein (CRABP) (3, 10), it was of interest to study the presence of this protein in melanoma cells. Cell cytosols were incubated with [^{3}H]all-trans-RA in the presence and absence of a 100-fold excess of non-labelled all-trans-RA, and after incubation the samples were analysed by Sephadex G-100 chromatography. The experiments indicated that melanoma cells contained only small amounts of specific CRABP.

Semiquantitative estimation gave approximately 0.3 fmol of CRABP/μg protein in melanoma cells (see also ref. 3). This is less than one tenth of the level of CRABP in human skin fibroblasts, when assayed by the same method (8).

Retinoids decrease interstitial collagenase activity in human skin fibroblasts (11, 12) and synovial cells (13). However, retinoids do not affect all proteases similarly, e.g. the activity of the elastase-like enzyme produced by skin fibroblasts has been shown to be increased by retinoids (12, 14). Thus it is possible that retinoids modulate the activities of various enzymes differently; the activity of certain enzymes being inhibited while some others may be stimulated or unaffected.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the expert technical assistance of Miss Heli Auno and Mrs Eeva Lehtimäki.

This study was supported in part by grants from the Finnish Academy of Sciences and Orion Corporation Ltd., Helsinki.

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