Variable Effects of Retinoids on Two Pigmenting Human Melanoma Cell Lines

Eileen Hoal, E. Lynette Wilson, and Eugene B. Dowdle
Department of Clinical Science and Immunology, University of Cape Town Medical School, Observatory, Cape Town, South Africa 7925

ABSTRACT

Two melanoma cell lines, each derived from a different patient with metastatic disease, were very similar in their appearance, their growth characteristics, and their tendency to differentiate and to pigment in culture as they become confluent. These lines, UCT-Mel 1 and UCT-Mel 2, were used to study the effects of retinoic acid and other derivatives of vitamin A. When added to UCT-Mel 1 cells, retinoids had only a modest effect on plasminogen activator release and were without measurable effect on morphology, growth, or tyrosinase synthesis.

In contrast, when added to UCT-Mel 2 cells, retinoids appeared to induce a more differentiated state evident as an inhibition of cell proliferation and the assumption of a dendritic morphology. Paradoxically, however, retinoids caused a striking inhibition of the density-dependent intracellular accumulation of tyrosinase and melanin that was taken to represent spontaneous in vitro differentiation.

Culture of UCT-Mel 2 cells in the presence of retinoic acid resulted in initial inhibition followed by marked stimulation of cellular plasminogen activator release. The data suggest that the manner in which retinoids exert their effects on cells in vitro does not depend on the histological origin of the tumor cells being studied but on the innate responsiveness of that particular cell line to the retinoid or compound in question.

INTRODUCTION

Melanoma cell lines provide useful models for the in vitro study of cellular differentiation since many of them express characteristics such as melanin synthesis and the dendritic morphology that are associated with the mature cellular phenotype. One might expect, therefore, that they would respond to biologically active compounds that are known to influence cellular proliferation and differentiation. Of the various agents that are of interest, retinoids occupy a prominent position.

Retinoids have been shown to be capable of reversing squamous metaplasia (18, 26) and of inhibiting carcinogenesis in experimental animals (2, 17, 19, 27, 31), and they have been used, with some success, for the treatment of actinic keratoses and basal cell carcinomas (3) and lung carcinoma (16) in man. This class of compounds has also been found to inhibit the growth of a number of tumor cell lines in vitro (12, 15, 21). On the other hand, retinoids may, in certain circumstances, induce activation of malignant cells (5, 10, 36), and they have been found to stimulate the growth of a human melanoma cell line (11).

Retinoids have therefore been shown to have both antitumor properties and the ability to enhance tumorigenesis.

A number of authors have recently reported experiments which demonstrate that retinoids enhance melanogenesis in melanoma cells (13, 15, 29). In this paper, we report our observations on the effects of retinoids on the morphology, pigmentation, and proliferation of 2 human melanoma cell lines cultured in vitro. Since plasminogen activator synthesis has been correlated with growth (4, 8) and cellular transformation (14, 20, 22, 33) and since synthesis of this enzyme by human cells has been shown to be modulated by retinoids (35), the effects of these compounds on the secretion of plasminogen activator were also studied. Although retinoids inhibited melanogenesis and stimulated plasminogen activator synthesis in one of the cell lines, these responses were somewhat complex in nature and were not observed in the other cell line.

MATERIALS AND METHODS

Cell Culture. Two human melanoma cell lines, UCT-Mel 1 and UCT-Mel 2, were used for this study. Both came from 67-year-old Caucasian females and were established from lymph node metastases taken at the time of surgery. UCT-Mel 1 was derived from an inguinal node and UCT-Mel 2 from an axillary node. The cell lines, which grow as adherent monolayers, were established and maintained using techniques described previously in detail (34).

In both cell lines, the intracellular content of tyrosinase is strikingly related to the density of the cultures, being high as the cultures reach confluence, falling to barely detectable levels within 24 hr of trypsinization and reseeding at a density of less than 5 x 10^5/60-mm Petri dish. These lines were maintained in Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum.

Unless otherwise stated, cells were seeded at 2.5 x 10^5 cells/60-mm plastic Petri dish or 1 x 10^5 cells/35-mm dish. Retinoids were added to the culture dishes 24 hr after seeding to give the desired final concentration. Medium, with or without freshly prepared retinoids, was replaced at 48-hr intervals.

Retinoids were dissolved in absolute ethanol to give final stock solutions of 10^-2 M from which dilutions were made.

Assay for Growth Inhibition. Cells were seeded at 10^5/35-mm Petri dish, and retinoids were added 24 hr later. At 48-hr intervals thereafter, cells in duplicate dishes were detached with 0.25% trypsin and counted on an electronic particle counter (Model ZBI Coulter Counter; Coulter Electronics, Inc., Hialeah, Fla.).

Assay for Tyrosinase. Cells for this assay were harvested by rinsing adherent monolayers with phosphate-buffered saline (containing, per liter, 800 mg of NaCl, 200 mg of KCl, 1150 mg of Na_2HPO_4, and 200 mg of K_HPO_4) followed by scraping with a plastic policeman. Cells were pelleted by centrifugation at 350 x g for 5 min. The pellet was...
duplicate cultures were counted. Acid was removed from a set of cultures (•). At 48-hr intervals, cells from containing 10⁻⁶ M retinoic acid (•) was added to cultures. On Day 8, retinoic acid gradually recovered to control levels when retinoic acid was over the 2-week period of the experiment and cell numbers to cytotoxicity since no decrease in cell viability was observed previously in detail (34).

**Assay for Melanin.** Melanin content was measured by the colorimetric method of Whittaker (32) as adapted by Lotan and Lotan (13) using synthetic melanin as a standard. Results are expressed as µg melanin per mg protein.

**Assay for Plasminogen Activator.** Plasminogen activator was assayed in serum-free harvest fluids collected from control and retinoid-treated cells by measuring the plasminogen-dependent release of soluble radioactive fibrin degradation peptides from insoluble [¹²⁵I]-fibrin-coated Linbro multiwell plates. This method has been described previously in detail (34).

**Assay for Lactate Dehydrogenase.** Lactate dehydrogenase, present in the same cell lysate as that prepared for the tyrosinase assay, was measured by the method of Kornberg (9).

**Reagents.** Materials were obtained from the following sources: plastic ware for cell culture from Falcon Plastics, Oxnard, Calif.; cell culture medium and fetal bovine serum from Grand Island Biological Co., Grand Island, N. Y.; Linbro multiwell plates (No. FB-16-24-TC) from Flow Laboratories Ltd., Irvine, Scotland; Na[¹²⁵I] (carrier free) from the Radiochemical Centre, Amersham, Buckinghamshire, England; retinoids, trypsin, tyrosine, and melanin from Sigma Chemical Co., St. Louis, Mo.

Human plasminogen and fibrinogen were prepared as described previously (34).

**RESULTS**

**Effect of Retinoic Acid on Growth.** Retinoic acid at 10⁻⁶ or 10⁻⁷ M had no effect on the growth rate of UCT-Mel 1. The growth of UCT-Mel 2 was inhibited by approximately 50% after 12 days culture in the presence of 10⁻⁶ M retinoic acid (Chart 1). No inhibition was observed with 10⁻⁷ M retinoic acid. The inhibition of growth seen with 10⁻⁶ M retinoic acid was not due to cytotoxicity since no decrease in cell viability was observed over the 2-week period of the experiment and cell numbers gradually recovered to control levels when retinoic acid was removed (Chart 1). Retinol and retinyl acetate at 10⁻⁶ M inhibited the growth of UCT-Mel 2 by approximately 30% after 12 days culture in the presence of these compounds.

**Effect of Retinoic Acid on Morphology.** The usual dendritic appearance of UCT-Mel 2 cells was accentuated by 10⁻⁶ and 10⁻⁷ M retinoic acid and, after 2 days culture in the presence of this compound, processes extended from almost all of the cells (Fig. 1). Retinol and retinyl acetate had a similar but less striking effect.

Retinoic acid at 10⁻⁶ M had no effect on the morphology of UCT-Mel 1 cells despite prolonged exposure to this compound. **Effects of Retinoids on Tyrosinase and Melanin Content.** Chart 2 summarizes the results of a typical experiment in which cells were seeded at low density after which they were cultured in the presence or absence of 10⁻⁶ M retinoic acid. After 12 days, retinoic acid was removed from one-half the cultures to which it had been added originally. Intracellular tyrosinase content was measured in duplicate dishes on Days 1, 12, and 19. It is evident from these data that, as UCT-Mel 2 cell density increased with the passage of time, so intracellular tyrosinase concentrations increased to reach approximately 0.9 units/µg protein on Day 12 and 1.1 units/µg protein on Day 19. When cells were cultured in the presence of 10⁻⁶ M retinoic acid, this intracellular accumulation of tyrosinase was strikingly inhibited. Removal of the retinoic acid from inhibited cultures on Day 12 was followed by a rapid increase in intracellular tyrosinase content, thus showing that inhibition by retinoic acid of tyrosinase induction is a reversible phenomenon. Similar results were obtained in 4 other experiments. In all cases, the experiments were so planned to ensure that, at the time of tyrosinase measurements, control and retinoid-treated dishes contained equal numbers of cells.

Retinoic acid had no effect on the morphology of UCT-Mel 2 cells despite prolonged exposure to this compound, processes extended from almost all of the cells. **Chart 1.** Effect of retinoic acid on growth in UCT-Mel 2 cells. Cells were plated at 1 x 10⁵/35-mm dish, and 24 hr later medium alone (A) or medium containing 10⁻⁶ M retinoic acid (B) was added to cultures. On Day 8, retinoic acid was removed from a set of cultures (C). At 48-hr intervals, cells from duplicate cultures were counted.

**Chart 2.** Effect of retinoic acid on tyrosinase in UCT-Mel 2 cells. Cells were plated at 1 x 10⁵/35-mm dish, and 24 hr later medium alone (A) or medium containing 10⁻⁶ M retinoic acid (B) was added to cultures. On Day 12, retinoic acid was removed from a set of cultures (C). Tyrosinase levels were determined on Days 1, 12, and 19.
and retinoic acid treated cultures, respectively. Levels of 0.72 ± 0.19 (S.D.) units/mg protein were obtained for untreated cultures. Intracellular lactate dehydrogenase levels could be measured. Levels of 0.65 ± 0.24 units/mg protein were obtained for untreated cultures. Intracellular lactate dehydrogenase levels were determined. In 6 experiments in which 10⁻⁶ M retinoic acid was present for 2 weeks, no significant effect on intracellular lactate dehydrogenase levels could be measured. Levels of 0.72 ± 0.19 (S.D.) and 0.65 ± 0.24 units/mg protein were obtained for untreated and retinoic acid treated cultures, respectively.

**Effects of Retinoids on Human Melanoma Cell Lines**

**Chart 3. Inhibition of tyrosinase in UCT-Mel 2 cells by retinoids.** Cells were plated at 2.5 x 10⁶/60-mm dish (control) or 5.0 x 10⁶/60-mm dish (retinoid treated), and 24 hr later medium alone or medium containing 10⁻⁶ M retinoic acid, 10⁻⁶ M retinyl acetate, or 10⁻⁶ M retinol was added to duplicate cultures. Fresh medium without or with retinoids was added at 48-hr intervals, and cells were harvested for tyrosinase assay after 14 days.

**Table 1** summarizes the results of a typical experiment in which cells were seeded at a sparse density after which they were cultured in the presence or absence of 10⁻⁶ M retinoic acid for 20 days. As can be noted, melanization in control cultures increased progressively with time in culture whereas retinoic acid-treated cultures failed to pigment.

**Effects of Retinoids on Plasminogen Activator.** Addition of 10⁻⁶ M retinoic acid to UCT-Mel 1 cells increased the rate of plasminogen activator secretion from a mean of 36 units/10⁶ cells/24 hr to 59 units/10⁶ cells/24 hr. The increase was apparent after 72 hr and could still be detected after 12 days.

When cultures of UCT-Mel 2 cells were exposed to 10⁻⁶ M retinoic acid, the effect on plasminogen activator release depended on the duration of the exposure to this retinoid. When enzyme secretion was measured 72 hr after addition of retinoic acid, inhibition to 30% of control levels was observed. Some inhibition was still apparent by the sixth day after which, despite the continued presence of retinoic acid, enzyme release recovered to control levels and by Day 18 exceeded control levels by approximately 8-fold. This biphasic response with initial inhibition followed by marked stimulation of enzyme release was consistently observed in 3 experiments and is shown in Chart 4. The secretion of plasminogen activator by UCT-Mel 2 cells did not vary with cell densities ranging from 1 x 10⁵ to 3 x 10⁶ cells/60-mm Petri dish and averaged 4 units/10⁶ cells/24 hr.

**Effect of Retinoic Acid on Lactate Dehydrogenase.** To establish whether or not the effects of retinoic acid were general in the sense that they affected multiple enzyme systems indiscriminately or whether they were relatively selective in their effects on tyrosinase and plasminogen activator synthesis, intracellular lactate dehydrogenase levels were determined. In 6 experiments in which 10⁻⁶ M retinoic acid was present for 2 weeks, no significant effect on intracellular lactate dehydrogenase levels could be measured. Levels of 0.72 ± 0.19 (S.D.) and 0.65 ± 0.24 units/mg protein were obtained for untreated and retinoic acid treated cultures, respectively.

**DISCUSSION**

The results of the studies that we present in this paper demonstrate the fact that 2 human cell lines derived from similar cancers and showing many similarities in vitro responded very differently to the effects of retinoids. Thus, while these compounds had only a modest effect on plasminogen activator release by UCT-Mel 1 cells and were without measurable effect on morphology, growth, and tyrosinase synthesis by these cells, they had pronounced effects upon all of these cellular functions in the case of UCT-Mel 2 cells.

When exposed to retinoids, UCT-Mel 2 cells proliferated less rapidly and assumed a dendritic morphology. In these respects, these cell lines paralleled the effects on tyrosinase.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment (days)</th>
<th>Cells/dish (10⁶)</th>
<th>Melanin content (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>1.06</td>
<td>8.0</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>7</td>
<td>1.07</td>
<td>7.7</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>2.50</td>
<td>12.2</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>12</td>
<td>1.95</td>
<td>7.1</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>2.74</td>
<td>18.9</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>16</td>
<td>2.17</td>
<td>5.3</td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
<td>3.18</td>
<td>28.3</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>19</td>
<td>2.64</td>
<td>7.2</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>2.91</td>
<td>38.3</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>20</td>
<td>2.69</td>
<td>5.8</td>
</tr>
</tbody>
</table>

**Chart 4. Effect of retinoic acid on plasminogen activator levels in UCT-Mel 2 cells.** Cells were plated at 2.5 x 10⁶/60-mm dish (control) or 5.0 x 10⁶/60-mm dish (retinoid treated), and 24 hr later medium alone or medium containing 10⁻⁶ M retinoic acid was added to duplicate cultures. Fresh medium with or without retinoids was added at 48-hr intervals, and cells were harvested for determination of melanin content at Days 7, 12, 16, 19, and 20.
are unable to pronounce with confidence on the general manner in which human melanoma cells can be expected to respond to retinoids.

Although it has only circumstantial bearing upon the data we present, the observation by Huberman et al. (7) is of interest. They found that the tumor promoter tetradecanoylphorbol acetate resulted in increased melanogenesis in melanoma cells. Had a simple relationship between tumor promotion and dedifferentiation been obtained, one would have expected the opposite result. This agent had no effect on tyrosinase levels in UCT-Mel 1 or UCT-Mel 2 cells.4

The manner in which retinoic acid treatment affected plasminogen activator release by UCT-Mel 2 cells is worthy of comment in a number of respects. In the first instance, an interesting “biphasic” response was observed in which a period of inhibition of plasminogen activator release was followed by a period of intense stimulation, in which retinoid-treated cells released enzyme at a rate that was 8-fold higher than that seen in corresponding untreated cells (Chart 4). This observation emphasizes the need for caution in interpreting results of experiments in which cellular responses to biologically active compounds are assayed. Such responses may be influenced by the duration of exposure to the compound in question or by the status of the culture at the time at which the response was measured. Although we have no explanation for the phenomenon, it clearly warrants further study for the understanding it may bring to the complex interactions between cell density, in vitro maturation, and the response to compounds that affect cellular differentiation.

In the second instance, our results may be compared with those of other workers who have shown that retinoids may either decrease (30) or increase (28, 35, 36) the rate of release of plasminogen activator. Generally speaking, the magnitude and the direction of the response have depended upon the cell type studied (36).

Since retinoids undoubtedly promote differentiation in many tissues, notably those of ectodermal origin, it was reasonable to expect that their use would contribute to the management of neoplasia and that in vitro systems could be used to establish their value in this regard. As indicated in a recent review of this subject by Schroder and Black (25), this expectation has only been realized in part, and the effects of retinoids on cultured cells in vitro have given cause for concern that they may aggravate or promote carcinogenesis. Our results would suggest that the manner in which retinoids and other agents exert their effects on cell lines in vitro depends less on the particular cell line to the compound in question.

ACKNOWLEDGMENTS

D. Fowlds and C. Fearns provided expert technical assistance.

REFERENCES

34. Wilson, E. L., and Dowdle, E. B. Secretion of plasminogen activator by normal, reactive and neoplastic human tissues cultured in vitro. Int. J.

Effects of Retinoids on Human Melanoma Cell Lines

Fig. 1. Morphological appearance of UCT-Mel 2 cells cultured in the absence (a) or presence (b) of $10^{-6}$ M retinoic acid for 48 hr. Marker, 50 µm. Phase contrast.
Variable Effects of Retinoids on Two Pigmenting Human Melanoma Cell Lines

Eileen Hoal, E. Lynette Wilson and Eugene B. Dowdle


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/12/5191

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.