Effects of Retinoids on Normal and Neoplastic Human Cells Cultured in Vitro

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ABSTRACT

The effects of retinoic acid on cultured human cells derived from normal and neoplastic tissues were studied. Retinoic acid consistently induced plasminogen activator synthesis by cells of mesenchymal origin (with the exception of adult skin fibroblasts) but not by cells of epithelial origin. The effect of retinoic acid was more pronounced than that of equimolar concentrations of retinol or retinyl acetate. Dexamethasone inhibited the retinoid-induced increase in plasminogen activator in lung- and foreskin-derived fibroblasts. Cells derived from normal or neoplastic tissues showed no consistent differences either in baseline rates of plasminogen activator release or in the magnitude of the retinoid effect.

INTRODUCTION

Retinoids (vitamin A and its congeners) have a number of important biological functions that establish this class of compounds as essential not only for photoreception but also for the normal development of ectodermal, mesenchymal, and endodermal tissues.

Of particular interest in this regard is the fairly general observation that retinoids have the capacity, both in vivo and in vitro, to control the growth and social behavior of cells and to promote their differentiation. Since neoplastic change is normally associated with aberrance in specialized cellular functions, this capacity of retinoids has been examined for its therapeutic potential for the management of established malignant disease or for its ability to reverse preneoplastic changes.

A number of studies have given encouraging results in this regard, and retinoids have been shown to reverse squamous metaplasia in organ culture (14), to inhibit the development of epithelial tumors in experimental animals (2, 7, 12, 15), to induce differentiation of teratocarcinoma cells in vitro (17), to suppress transformation induced by sarcoma growth factor (19), and to inhibit the induction of ornithine decarboxylase activity by the potent tumor promoter TPA² (22).

On the other hand, in a limited number of cases, retinoids have shown effects that mimic those of tumor-promoting phorbol esters (4, 9, 10, 27) and that would, on circumstantial grounds, disqualify them as candidates for the therapy of human neoplasia.

In view of these apparent conflicting reports and since most experiments to date have used animals or avian tissues for the in vitro study of retinoid action, we have undertaken a systematic survey of the effects of vitamin A derivatives on cultured human cells derived from normal and neoplastic tissues.

We have used plasminogen activator synthesis as the principal quantitative criterion for evaluating effects of retinoids on cultured cells. The main justification for doing so rests upon the fact that plasminogen activator synthesis is a modulatable cellular function requiring transcription of new mRNA for both induction and suppression (11, 20). Measurement of this enzyme therefore provides a convenient experimental means for establishing effects of test compounds upon genetic regulatory processes. Furthermore, induction of plasminogen activator synthesis has been correlated with expression of the malignant phenotype in cultured avian and animal cells (3, 5, 8, 20) and with effects of the cocarcinogen TPA (25, 28).

MATERIALS AND METHODS

Materials were obtained from the following sources: plastic ware for cell culture from Falcon Plastics, Oxnard, Calif.; cell culture media and fetal bovine serum from Grand Island Biological Co., Grand Island, N. Y.; Liniro multiwell plates (No. FB-16-24-TC) from Flow Laboratories, Ltd., Irvine, Scotland; sodium ¹²⁵I (carrier free) from Radiochemical Centre, Amersham, Buckinghamshire, Enalngd; disopropylfluorophosphate and retinoids from Sigma Chemical Co., St. Louis, Mo.; and human urokinase from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan.

Human plasminogen, protease- and inhibitor-free bovine serum albumin, and fibrinogen were prepared as described previously (26).

Human Tissues and Cell Cultures. Human cell cultures were derived from tissue samples obtained at the time of elective surgery or from cadaver transplant donors and were processed as described previously in detail (26). Human esophageal carcinoma cell cultures were generously provided by K. Robinson, University of Natal, Durban, South Africa.

Duplicate Petri dish cultures were studied according to a standard protocol in which subconfluent adherent monolayers were exposed for 48 hr to retinoids at concentrations varying from 0 to 10⁻⁶ M in Dulbecco’s modified Eagle’s medium or Roswell Park Memorial Institute Medium 1640 supplemented with 15% fetal bovine serum. The monolayers were then washed with warm Dulbecco’s modified Eagle’s medium or Roswell Park Memorial Institute Medium 1640 to remove serum, covered with serum-free medium containing retinoids at the same concentration as formerly present and incubated for a further 24 hr. The medium was then collected, centrifuged to remove debris, and stored frozen at -80° as described previously (26).

Plasminogen Activator Assay. Plasminogen activator released into the 24-hr harvest fluid was measured using a solid-phase ¹²⁵I-fibrin assay (16) in which plasminogen-dependent release of radioactive fibrin degradation peptides was related to that observed using urokinase standards prepared from calibrated commercial enzyme and assayed concurrently (26).

¹ To whom requests for reprints should be addressed. ² The abbreviation used is: TPA, 12-O-tetradecanoylphorbol-13-acetate.

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The commercial enzyme was supplied with the enzyme activity specified in Ploug units, and these units are used in this paper. Rates of enzyme synthesis were calculated as urokinase units/10⁷ cells/24 hr with a 95% error bound of ±20% of the mean. Analysis of the results obtained when 200 paired cultures were studied according to the protocol described has shown that duplicates differed, on average, by an amount corresponding to 26% of the duplicate mean with an upper 95% confidence limit of 40%.

RESULTS

Retinoic acid had no effect on cell numbers, viability, or morphology over the 72-hr time course of the experiments we report in this paper.

In all cases, with the exception of adult skin fibroblasts, retinoic acid increased the rate of plasminogen activator synthesis by cells of mesenchymal origin (Chart 1). This effect was found in mesenchymal cells of both normal and malignant origin and varied in magnitude from a 3- to a 115-fold increase over control levels.

The effects of retinoic acid on cells of epithelial origin were less consistent and less striking. In the case of normal epithelial cells derived from esophagus, bladder, kidney, and thyroid, retinoic acid either had no effect on plasminogen activator synthesis or induced a marginal increase (up to 1-fold). In one culture of normal kidney epithelium, the retinoid decreased activator release to 50% of control levels. Of 11 malignant epithelial cell cultures, several were inhibited by the retinoid, 3 were minimally stimulated, and one was unaffected (Chart 1).

There were no consistent differences between cells derived from normal or neoplastic tissues, either in base line rates of plasminogen activator release (Chart 2) or in the magnitude of their response to retinoids (Chart 1).

In many cases, significant effects of retinoic acid could be detected at concentrations of 10⁻⁷ and 10⁻⁸ M and occasionally at 10⁻⁹ M, but these were invariably less than those seen at 10⁻⁶ M (Table 1). As observed in the case of chick embryo fibroblasts (27), the effects of retinoic acid on human foreskin fibroblasts were more pronounced than those of equimolar concentrations of retinol or retinyl acetate (Chart 3). The response to retinoic acid could be completely blocked by simultaneous addition of actinomycin D (1 μg/ml) to the cultures. Dexamethasone at 10⁻⁶ M could partially or totally inhibit the retinoid-mediated increase in plasminogen activator levels in lung and foreskin fibroblasts (Table 2). The degree of inhibition was dependent on the concentration of retinoid added. Dexamethasone was less effective in retinoic acid than in retinyl acetate.

<table>
<thead>
<tr>
<th>Plasminogen activator (units/10⁷ cells/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 1.0 10 100 1000</td>
</tr>
</tbody>
</table>

Normal Epithelial

Malignant Epithelial

Table 1

The effect of retinoic acid on plasminogen activator secretion

<table>
<thead>
<tr>
<th>Retinoic acid concentration (M)</th>
<th>Plasminogen activator (units/10⁷ cells/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult lung fibroblasts</td>
<td></td>
</tr>
<tr>
<td>Specimen 1</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>405</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>280</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>54</td>
</tr>
<tr>
<td>10⁻⁹</td>
<td>18</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>0</td>
</tr>
<tr>
<td>10⁻¹¹</td>
<td>13</td>
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<tr>
<td>Foreseen fibroblasts</td>
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</tr>
<tr>
<td>Specimen 1</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>193</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>46</td>
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<tr>
<td>10⁻⁸</td>
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<td>6</td>
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<td>3</td>
</tr>
<tr>
<td>10⁻¹¹</td>
<td></td>
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<tr>
<td>Breast fibroblasts</td>
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</tr>
<tr>
<td>Specimen 1</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>54</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>30</td>
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<tr>
<td>10⁻⁸</td>
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<td>10⁻⁹</td>
<td>4</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>3</td>
</tr>
</tbody>
</table>

Chart 2. Plasminogen activator secretion by human cells cultured in vitro. Each point depicts the mean rate of plasminogen activator release by duplicate control cultures used in the experiments summarized in Chart 1. As in Chart 1, open circles depict results obtained with adult skin fibroblasts.
methasone also inhibited the basal levels of activator secreted by these cells in the absence of retinoid.

**DISCUSSION**

The results we report in this study show clearly that the effects of retinoids on plasminogen activator secretion by cultured human cells were related to the tissues from which the cultures were established. Retinoic acid markedly stimulated the production of this enzyme by cells of mesenchymal origin but had little similar effect on cells of epithelial origin. The literature dealing with the action of retinoids has tended to emphasize the effects of these compounds on epithelial cells (2, 7, 12, 14, 15) with little attention to possible direct effects of the vitamin on mesenchymal tissues. To the best of our knowledge, the observations we report in this paper represent the first documentation of in vitro differential responsiveness of human cells to retinoids.

As indicated by our present data (Chart 2) and our previously reported findings (28), the rate of plasminogen activator synthesis by human cells cultured in vitro is not reliably related to the neoplastic status of the tissue from which the cells were derived. One would, therefore, tend to regard plasminogen activator release as a poor specific indicator of the malignant phenotype in human cells and hence of uncertain significance in the context of retinoid therapy of human neoplasia. On the other hand, the differences we have observed between the effect of retinoids on epithelial and mesenchymal cells as far as activator synthesis is concerned may extend to the genetic regulation of differentiation, and the possibility cannot be ignored that retinoids, while preventing tumors of epithelial origin (2, 7, 12, 14, 15), may function as promoters of tumorigenesis in mesodermal tissues. It may be noted, in this regard, that fibroblasts were used to show synergism between the effects of retinoids, TPA, and Rous sarcoma virus (27). The evidence is, however, at present circumstantial, and it would be of interest to observe the effects of retinoids on experimental mesodermal tumors, particularly in view of the report by Meriman and Bertram (6) indicating that retinoids reversibly inhibited transformation in a mouse fibroblast cell line.

The value of plasminogen activator induction as a means for detecting differential susceptibility of apparently similar tissues to the action of biologically active compounds is illustrated by the fact that fibroblasts from adult skin, although by morphological and other gross criteria identical to fibroblasts derived from foreskin, lung, or other tissues, were distinguished by their failure to respond to retinoids with an increase in plasminogen activator production.

It is also of interest that the induction of plasminogen activator synthesis by retinoic acid was inhibited by the antiinflammatory steroid dexamethasone. This class of compounds has been shown to have a marked inhibitory effect on carcinogenesis and has been shown to effect both tumor initiation and promotion (1, 13, 18). In addition, a relationship between the potency of a series of antiinflammatory steroids for the inhibition of tumor promotion and the ability of these steroids to inhibit plasminogen activator has been documented (23).

The base line secretion of plasminogen activator was also inhibited by dexamethasone. Dexamethasone has been shown to have similar inhibitory effects on the enzyme secreted by mouse macrophages and rat hepatoma cells (21, 24).

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**REFERENCES**

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