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Journal
Prostaglandins, 26(3)

ISSN
0090-6980

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Publication Date
1983-09-01

DOI
10.1016/0090-6980(83)90179-x

IN VITRO MODULATION OF HUMAN AND MURINE MELANOMA GROWTH BY PROSTANOID ANALOGUES

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ABSTRACT

The inhibitory effect of various prostaglandin analogues on the anchorage independent growth of murine and human melanoma cells was measured. PGA analogues (which were modified at C-16 and C-18) did not demonstrate any major improvement in activity over PGA alone. These included 16,16-dimethyl PGA_1, 16,16-dimethyl-PGA_2, 16,16-dimethyl-18-oxa-PGA_2 and trans-Δ-2-15-α acetoxy-16,16-dimethyl-18-oxa-11-deoxy-PGE_1-methylester. The thromboxane synthetase inhibitor, U51605, demonstrated weak anti-proliferative activity. PGD_2 (with a ketone at C-11 versus C-9 for PGA and PGE) was the most potent prostaglandin tested. Cells from melanoma lines displayed species differences in their sensitivities. PGA_1 and PGE_1 were the most potent inhibitors of the anchorage independent growth of murine melanoma cells. On human melanoma cells PGD_2 was the most active prostaglandin, 2-3 times more potent than PGA_1; PGE_1 was a very weak inhibitor.

INTRODUCTION

Prostaglandins, especially the A series (1-3), are potent modulators of animal tumor cells, both in vitro (1-5) and in vivo (6-10). We have recently reported on the use of the bilayer soft agar assay to measure the effect of prostaglandins on anchorage-independent growth (4). The A and E series were potent inhibitors of murine melanoma growth. The anchorage-independent growth of melanoma cells obtained directly from patients and human melanoma cell lines was also arrested by PGA_1 (11). The major problem in extending these observations to the clinic is that the concentrations and exposure times needed to produce irreversible inhibition of growth with these prostaglandins as single agent effectors would be excessively toxic for patients (12). With this in mind, structural analogues of PGA_1 were investigated to determine their activity.

MATERIALS AND METHODS

Prostaglandins

PGA_1, PGE_1 and PGD_2 were purchased from Upjohn Diagnostics (Kalamazoo, Mich.). PGA_2 was obtained from Sigma (St. Louis, Mo). 16,16,Dimethyl-PGA_2, U39770; 16,16-dimethyl-PGA_1, U42416; and 9,11-diazoprosta-5,13-dienoic acid, U51605 were a generous gift from Dr. John Pike, The Upjohn Company (Kalamazoo, Mich.). 16,16,-Dimethyl-18-oxa-PGA_2, HR466; and trans-Δ 2-15a--acetoxy-16,16-dimethyl-18-oxa-11-deoxy-PGE_1-methyl ester , HR601 were a generous gift from Drs. W. Bartmann, G. Beck and M. Schorr, A.G. Hoechst (Frankfurt, W. Germany). All prostaglandins with the exception of HR466 and HR601 were dissolved in ethanol at a concentration of 10

mg/ml. HR466 and HR601 were supplied dissolved in ethanol at a concentration of 1 mg/ml in sealed ampules. These reagents were diluted in complete culture media to obtain final concentrations. Appropriate ethanol controls were included for all experiments.

Melanoma cells and soft agar assay

The origin of the human melanoma cell strain C8146c and murine melanoma cell line CCL 53.1 and conditions for growth in soft agar have been previously described (4,11). PG were added with the cells to the upper layer for these continuous contact experiments. MIRW5 was cloned from the MIRW human melanoma developed in this laboratory (13). The MIRW5 cell line was injected into nude mice and the tumor removed 5 weeks later and subcultured in Ham's F-10 containing 10% fetal calf serum. Cells from the first four subcultures were used in the experiments presented.

RESULTS

Prostaglandin effects on the anchorage independent growth of murine melanona cells

We tested the effects of several PGA-like analogues in vitro on the growth of murine melanoma cells in soft agar. Table 1 ranks the effectiveness of these prostaglandins to inhibit anchorage-independent growth relative to PGD_2. The ID_{50} for each PG was extrapolated from the dose response curves obtained over the 0.1 to 10 μg/ml concentration range and ranked relative to the ID_{50} for PGD_2. PGD_2 was assigned a rank of one for each cell line. The higher the ranking the more potent the PG was in inhibiting anchorage-independent growth. All the PGA-like analogues were less potent than PGA_1. The more stable analogue, 16,16-dimethyl-PGA_1, was only one-fourth as potent as PGA_1 in inhibiting murine melanoma growth. PGA_2 had very little activity, which corresponded to its effect on human melanoma cells (11). The more stable 16,16-dimethyl analogue, U39770, was significantly more active than PGA_2. These analogues retained the ability to totally inhibit the anchorage-independent growth of murine melanoma. The 18-oxa derivative of 16,16-dimethyl PGA_2,HR466, did not demonstrate any change in activity in comparison to U39770 and was relatively weak in comparison to PGA_1. U51605, a thromboxane A_2 synthetase antagonist (14), has been shown to inhibit the monolayer

TABLE 1

POTENCY OF PROSTAGLANDINS ON MELANOMA COLONY FORMATION

ALL RELATIVE TO ID_{50} FOR PGD_2[a,b]

	HUMAN MELANOMA		MURINE MELANOMA
	C8146c	MIRW5	CCL 53.1
PGD_2	1.00	1.00	1.00
PGA_1	0.52	0.29	1.91
U42416	0.46	0.31	0.53
U39770	0.45	0.50	0.32
PGA_2	0.25	0.11	0.13
HR466	0.24	0.11	0.32
PGE_1	0.16	0.08	1.54
U51605	0.15	0.08	0.06
HR601	NE[c]	NE	NE

[a]Inhibitory potency of PG ranked relative to ID_{50} for PGD_2, CCL 53.1, 1.05 μg/ml; C8146c, 0.45 μg/ml; and MIRW5, 0.80 μg/ml.

[b]C8146c yielded 2860 ± 30 colonies per 15,000 cells; MIRW5 yielded 2104 ± 136 colonies per 10,000 cells; and CCl 53.1 yielded 3056 ± 52 colonies per 5,000 cells.

[c]NE - no effect on colony formation up to maximum concentration of 4 μg/ml.

growth of B-16a murine melanoma cells (5). However, this analogue did not appreciably inhibit the growth of murine melanoma cells in soft agar (Fig. 1). Even at high concentrations (10 μg/ml) only a 15% reduction in colony number was observed. Another analogue HR601 which is in the fairly stable trans-Δ2-11-deoxy-PGE_1 series and is equipotent to HR466 in vasodepressor activity (15) had no effect on the anchorage-independent growth of murine melanoma cells. PGD_2 which unlike PGA or PGE has an 11-oxa, was about one-half as potent as PGA_1 (Fig. 1).

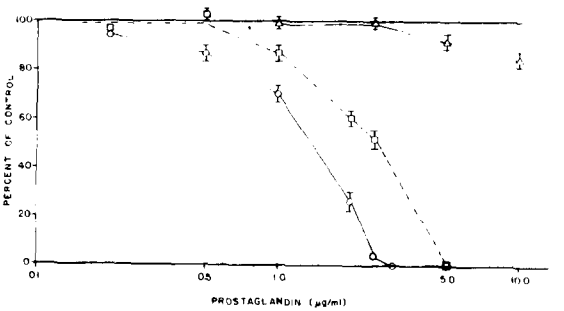

Figure 1 Sensitivity of murine melanoma to PGA_1 (○–○), PGD_2 (□–□) and U51605 (△–△), 5,000 murine melanoma cells were plated in triplicate and controls yielded 3056 ± 52 colonies greater than 60 microns on day 10. The experiment was repeated with similar results.

Prostaglandin effects on the anchorage-independent growth of human melanoma cells

PGA_1 inhibited the growth of human melanoma cells in soft agar in a dose dependent manner (11). We measured the effect of several prostanoid analogues on the growth of cells from two human melanoma lines in soft agar (Table 1). C8146c melanoma cells were not as sensitive to PGA_2. Both 16,16-dimethyl PGA were 88% as potent as PGA_1 in the inhibition of growth as determined by colony formation. The 18-oxa derivative, HR466 was one-half as potent as U39770. HR466 still retained full agonistic properties effecting a ninety-percent reduction in colony formation at 4 µg/ml. As we had observed previously with two other human melanoma cell lines (11), PGE_1 was a relatively weak inhibitor of anchorage independent growth. The di-azido prostanoid, U51605, was a very weak inhibitor of anchorage independent growth. The maximum inhibition observed was a 25% reduction in colony-formation at 5 µg/ml. HR601 did not effect the growth of C8146c cells to a maximum concentration of 4 µg/ml. PGD_2, with the carbonyl at C-11, was a very strong inhibitor of human melanoma anchorage-independent growth. Complete inhibition of colony formation was obtained with a PGD_2 concentration of 1 ug/ml versus the 2.5 µg/ml needed with PGA_1.

The MIRW5 human melanoma cell strain was not as sensitive to PGA_1 (Table 1). It took twice the concentration of PGA_1 to produce an inhibition comparable to the effect on C8146c. PGA_2 had only one-third the potency of PGA_1. The dimethyl derivatives, U39770 and U42416, were very potent inhibitors of anchorage independent growth. The PGA_1 analogue was about as effective as PGA_1, whereas U39770 was from 1.6-fold more potent than PGA_1. However, 16,16-dimethyl-18-oxa-PGA_2 was only one-fifth as potent as 16,16-dimethyl-PGA_2. As was observed for cells from the C8146c cell line, HR601 was also inactive. U51605 had no significant effect on the growth of these cells in soft agar. As was the case with C8146c cells, PGD_2 was the most active prostaglandin tested. It was 3-fold more potent than PGA_1 for the inhibition of MIRW5 growth in soft agar.

DISCUSSION

PGD_2 was the most potent prostaglandin we have tested on human melanoma cell growth in soft agar; it also was active on murine melanoma cells. The direct anti-proliferative effects on murine melanoma may explain some of its previously observed anti-metastatic properties which have been attributed to the inhibition of platelet aggregation (10). Recently PGD_2 was shown to exert a dose-dependent inhibition of the growth of cells from human leukemia (16) and neuroblastoma lines (17). PGD_2 has a ketone at C-11 whereas PGA and PGE have the ketone at C-9. PGF which does not have a ketone also was inactive (4,11,17). The presence and position of the ketone in the cyclopentane ring is not the only determinant for activity, since PGB_1 was relatively inactive (11). PGB_1 may represent a special

case because the position of the double bond in the cyclopentane ring fixes the molecular conformation into a unique secondary structure (18). Experience to date suggests that the relationship and type of substitutions at C-9 and C-11 are important for inferring anti-tumor activity to the prostaglandin and suggests that other structural analogs of the cyclopentane ring should be tested.

The 16,16-dimethyl-PGA analogs were as active as PGA_1 in inhibiting anchorage independent growth of human melanoma cells. However, they had sharply reduced activity on the murine melanoma cells. Though the 16,16-dimethyl modification did not greatly enhance the observed potency of these analogues in vitro, one would predict because of their increased stability that U39770 and U42416 would be more active in vivo. However, conversion of C-18 to oxygen greatly reduced activity and suggested that 18-oxa derivatives need not be tested further.

HR601 was totally inactive on the growth of cells from the three melanomas tested. Since the 18-oxa modification sharply reduced the activity of 16,16-dimethyl-PGA_2 (Table 1), one can not ascribe the lack of activity to Δ-2, 11-deoxy and/or 15 α-acetoxy modifications. Further testing with simpler 11-deoxy-PGE_1 and Δ-2,11-deoxy-PGE_1 is needed before one can assign structure-function relationships. It is worthy that HR601 was biologically active in other in vitro systems and was very active on antihypertensive dogs (16).

It is difficult to draw absolute conclusions from the results with the thromboxane A_2 synthetase inhibitor U51605 (14), and the Δ-2-11-deoxy-PGE_1 derivative, HR601, because of the multiple modifications. For example, with U51605 one does not know if weak anti-tumor activity was due to the 9,11-azo or the 15-deoxy. Honn and Meyer found this analogue inhibited the growth of B16a, a murine melanoma cell line (5). U51605 had negligible effect on the growth of cells from the murine cell line (CCL 53.1) used here. One should note we are measuring anchorage independent growth in a semi-solid media, whereas the previous investigation measured mono-layer growth in a liquid media. U51605 did demonstrate some inhibitory activity on one of the human melanoma cell lines, Table 1. So there may be heterogenity in the sensitivity of melanomas to this analogue.

An important and disturbing result was the differences in sensitivity between the cells from murine CCL 53.1 and the human melanoma cell lines. On murine melanoma cells PGA_1 and PGE_1 were equal in potency (11) and more potent than PGD_2, which had significantly greater activity than PGA_2 (Fig. 1). Human melanoma displayed a completely different sensitivity, with PGD_2 being more potent than PGA_1 which in turn was greater than PGE_1 and PGA_2. These results suggest caution should be exercised in transposing prostaglandin data in murine systems to predict results or activity in human cell lines.

ACKKNOWLEDGMENTS

Supported in part by grants from the National Cancer Institute (CA17094, CA27502) and American Cancer Society (PDT184). We thank L. Kimball for preparation of the manuscript and J. Buckmeier for preparation of the graphics. We also thank E. Peters and D. Sander for their excellent technical assistance.

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Editor: Bernard Jaffe
Received: 3-4-83
Accepted: 6-27-83