PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 562–567 (1981)

Prostaglandin Analogs as Inhibitors of Tumor Cell DNA Synthesis[1] (41109)

KENNETH V. HONN, MARY ROMINE, AND ANNE SKOFF

Departments of Radiology, Radiation Oncology and Biological Sciences, Wayne State University, Detroit, Michigan 48202

Abstract. Analogs of the A and E series prostaglandins were screened *in vitro* for their chemotherapeutic potential against Lewis lung carcinoma and B16 amelanotic melanoma cells, since *in vivo* studies had demonstrated that PGA_1 and an analog of PGE_2 inhibited tumor growth. DNA synthesis by collagenase-dispersed tumor cells was evaluated at timed intervals after exposure to the 16, 16-dimethyl analogs of PGA_1, PGA_2, PGE_1, and PGE_2. In general, the PGA derivatives were more potent than the PGE analogs. However, the maximum effect was achieved with combined PGA and PGE treatment. These findings demonstrate that A and E series prostaglandin analogs inhibit DNA synthesis in two unrelated tumor types and thus may be potent inhibitors of tumor growth. These effects were not due to tumor cell cytotoxicity. These results suggest that these compounds may prove useful as cancer chemotherapeutic agents.

Numerous drugs currently used for the chemotherapy of cancer exhibit cytotoxicity and mutagenicity (1). This has prompted a search for natural inhibitors or their analogs which may prove effective in the control of growth of malignant tumors. Considerable research effort has centered around the retinoids, analogs of vitamin A, which have demonstrated anticarcinogenic and antitumor effects (2, 3). Another group of naturally occurring bioregulatory compounds with wide tissue and species distributions (4) are the prostaglandins.

Prostaglandins (PG's) have been shown to affect normal (5) and neoplastic (6) cell proliferation. Dimethyl analogs of PGE_2 have been reported to decrease tumor growth (7) and induce neoplastic cell differentiation (8). We have demonstrated that the A series PG's (PGA_1, PGA_2) were potent inhibitors of Harding–Passey melanoma cell DNA synthesis. PGA_1 and PGA_2 were more potent inhibitors of melanoma cell DNA synthesis than equivalent doses of the chemotherapeutic agents, adriamycin and hydroxyurea (9). This inhibition of DNA synthesis was not related to cytotoxicity based upon vital dye exclusion (9). In the present study, we have compared the antitumor effects of the stable (16,16-dimethyl) analogs of PGA and PGE series compounds in two tumor lines. In addition, possible synergistic action between A and E series prostaglandin analogs was investigated.

Materials and Methods. The B16 amelanotic melanoma ($B16_a$) and Lewis lung carcinoma (3LL) were originally obtained from the DCT-Animal and Human Tumor Bank. Subcutaneous tumors were maintained in male syngeneic C57BL/6J mice (Jackson Laboratories). All the studies outlined below utilized monodispersed cells obtained from primary subcutaneous tumors by a modification of our described procedure (9). Aseptically removed subcutaneous tumors were diced and placed in sterile Eagle's minimum essential medium (MEM) buffered with sodium bicarbonate (15 m*M*) and Hepes (25 m*M*). MEM used for tumor cell dispersion contained collagenase type III (Worthington, 1 mg ml^{-1}), DNase I (Sigma, 50 μg ml^{-1}), soybean trypsin inhibitor (Worthington, 100 μg ml^{-1}), and fatty acid free human serum albumin (Sigma, 10 mg ml^{-1}). Cells were dispersed (for 30 min and then for 60 min, 37°) under air in a Dubnoff metabolic shaker (90 oscillations min^{-1}). Supernatants were collected through cheesecloth, centrifuged (100*g*, 10 min), and pellets were washed twice and resuspended in MEM buffered as

[1] This work was supported by the Detroit General Hospital Research Corporation.

0037-9727/81/040562-06$01.00/0

described above. Final preparations consisted of monodispersed (>99%) cells of high viability (>95%) with low (4–7%) host stromal cell contamination. Cells were resuspended at a concentration of 5×10^5 ml^{-1} and incubated in MEM without serum supplement. The effects of 16,16-dimethyl-prostaglandin A_1, A_2, E_1, and E_2 (di-Me-PGA_1, di-Me-PGA_2, di-Me-PGE_1, di-Me-PGE_2) on [^{3}H]thymidine (2 μCi ml^{-1}; 4 μCi 10^6 cells^{-1}; New England Nuclear, 45.2, 48.0, 57.0, or 80 Ci mmole^{-1}; 1 Ci = 3.7×10^{10} Bq) uptake and incorporation into DNA were examined at timed intervals. The procedure for estimating the acid-insoluble (incorporation) and acid-soluble (uptake) thymidine pools have been described (9). In general, the analogs were dissolved in ethanolic (1.0%) sodium carbonate (0.02%) at concentrations of 2.5, 1.0, or 0.1 mg ml^{-1}. Ethanolic sodium carbonate was used as the control, yielding a final ethanol concentration of 0.01% (v/v). For the synergism experiment the analogs were dissolved in ethanolic (1.0%) sodium carbonate (0.02%) at a concentration of 1.0 mg ml^{-1} and were serially diluted to 0.5, 0.4, and 0.2 mg ml^{-1}. Controls were treated as described above. Data are expressed as moles of thymidine 10^6 cells^{-1} or as percentage control. A minimum of four replicate incubations was used per datum point. Data were analyzed by analysis of variance and Students t test. Differences were accepted as significant when $P \leq 0.05$.

Results. Suspensions of $B16_a$ and 3LL tumor cells steadily incorporated [^{3}H]-thymidine into DNA over an 18-hr incubation period, (Table I). 3LL cells *in vitro* incorporated more [^{3}H]thymidine into DNA than $B16_a$ cells (Table I), consistent with the greater *in vivo* and *in vitro* doubling time of the former (10). Uptake of [^{3}H]thymidine increased during the first hour but remained plateaued thereafter. The effects of the dimethyl prostaglandin analogs on this normal pattern of tumor cell DNA synthesis were evaluated during a 4- and 18-hr incubation period.

In the Lewis lung carcinoma, incubation with each of the di-Me-PG's depressed thymidine incorporation into DNA at 4 hr over a dose range of 10–25 μg ml^{-1}. The analogs were ineffective at this time interval at a dose of 1 μg ml^{-1}. Di-Me-PGE_2 was approximately 30% less effective than the other analogs in this inhibition (Table II). At 18 hr of incubation, each of the di-Me-PG's had inhibited DNA synthesis in 3LL cells by at least 50% (Fig. 1). At the maximal dose di-Me-PGE_2 was slightly less effective than the other dimethyl analogs examined, depressing thymidine incorporation by 72.9% below controls in comparison to di-Me-PGA_1, di-Me-PGA_2, and di-Me-PGE_1 which depressed DNA synthesis by 86.3%, 89.2%, and 82.6%, respectively (Fig. 1). At each time interval examined, a dose response to all of the di-Me-PG's was observed (Table II, Fig. 1). Monitoring of cellular uptake of [^{3}H]thymidine indicated that inhibition of DNA synthesis by PG

TABLE I. INCORPORATION OF [^{3}H]THYMIDINE BY LEWIS LUNG CARCINOMA AND B16 AMELANOTIC MELANOMA

Hours	Lewis lung carcinoma[a]	$B16_a$ melanoma[b]
1	0.15 ± 0.04[c]	0.08 ± 0.01
2	1.90 ± 0.15	0.29 ± 0.03
4	4.14 ± 0.12	2.30 ± 0.03
8	7.75 ± 0.74	3.14 ± 0.11
18	16.2 ± 0.16	4.61 ± 0.22

Note. Cells (5×10^5 ml^{-1}) were exposed to 2 μCi ml^{-1} [^{3}H]thymidine for the above time intervals.

[a] Data are presented as picomoles of thymidine ± SEM per 10^6 cells; $n = 8$.

[b] Specific activity of [^{3}H]thymidine = 57.0 Ci mmole^{-1}.

[c] Specific activity of [^{3}H]thymidine = 48.0 Ci mmole^{-1}.

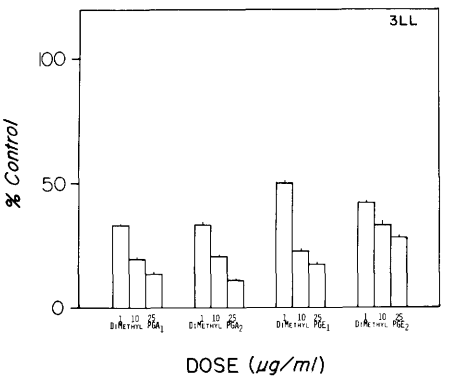

FIG. 1. Dose-dependent inhibition of 3LL tumor cell DNA synthesis by di-Me-PG analogs after 18 hr incubation. Refer to Table II for experimental details.

TABLE II. EFFECTS OF 4-hr EXPOSURE TO A AND E SERIES PROSTAGLANDIN ANALOGS ON TUMOR CELL DNA SYNTHESIS

Treatment	Lewis lung carcinoma		$B16_a$ melanoma	
	pmoles thymidine	% Control	pmoles thymidine	% Control
Control	4.14 ± 0.06[a]		1.62 ± 0.05[a]	
Dimethyl PGA_1				
1.0 $\mu g\ ml^{-1}$	3.92 ± 0.13	94.8 ± 1.6[b]	1.43 ± 0.05	88.0 ± 1.4[b]
10.0 $\mu g\ ml^{-1}$	2.54 ± 0.04	61.3 ± 0.5	0.50 ± 0.03	29.9 ± 0.8
25.0 $\mu g\ ml^{-1}$	2.07 ± 0.03	50.0 ± 0.4	0.39 ± 0.01	23.7 ± 0.4
Dimethyl PGA_2				
1.0 $\mu g\ ml^{-1}$	4.11 ± 0.02	99.4 ± 1.8	1.24 ± 0.10	76.3 ± 3.0
10.0 $\mu g\ ml^{-1}$	2.67 ± 0.04	64.6 ± 0.5	0.49 ± 0.08	30.2 ± 2.5
25.0 $\mu g\ ml^{-1}$	1.83 ± 0.05	44.2 ± 0.7	0.52 ± 0.06	31.9 ± 1.8
Dimethyl PGE_1				
1.0 $\mu g\ ml^{-1}$	4.23 ± 0.11	102.3 ± 1.4	1.73 ± 0.03	106.3 ± 0.8
10.0 $\mu g\ ml^{-1}$	2.57 ± 0.10	62.0 ± 1.2	0.62 ± 0.01	38.1 ± 0.2
25.0 $\mu g\ ml^{-1}$	2.26 ± 0.12	54.5 ± 1.5	0.24 ± 0.02	14.7 ± 0.6
Dimethyl PGE_2				
1.0 $\mu g\ ml^{-1}$	4.51 ± 0.06	109.0 ± 0.7	1.65 ± 0.03	101.6 ± 0.9
10.0 $\mu g\ ml^{-1}$	3.90 ± 0.10	94.1 ± 1.2	1.34 ± 0.09	82.5 ± 2.6
25.0 $\mu g\ ml^{-1}$	3.42 ± 0.17	82.8 ± 2.1	1.01 ± 0.09	62.4 ± 2.5

Note. The cells ($5 \times 10^5\ ml^{-1}$) were exposed to analogs, at the concentrations specified simultaneously with [^{3}H]thymidine (2 μCi ml^{-1}). The specific activity of [^{3}H]thymidine was 45.2 Ci $mmole^{-1}$ for $B16_a$ experiment and 57.0 Ci $mmole^{-1}$ for Lewis lung.

[a] Data are presented as picomoles of thymidine ± SEM per 10^6 cells; $n = 4$ for experimental groups; $n = 8$ for controls.

[b] Data are present as percentage of control ± SEM.

analogs was not due to interference with [^{3}H]thymidine uptake.

PGA and PGE analogs depressed DNA synthesis by dispersed $B16_a$ cells. After 4 hr incubation, di-Me-PGA_1 and di-Me-PGA_2 at doses of 1–25 $\mu g\ ml^{-1}$ significantly depressed $B16_a$ melanoma DNA synthesis (Table II). Di-Me-PGE_1 and di-Me-PGE_2 were effective at the two higher doses examined (Table II). In 3LL tumor cells treated with the PGA and PGE analogs, there was a significantly greater inhibition of DNA synthesis at 18 hr than at 4 hr. In contrast, inhibition of $B16_a$ DNA synthesis after an 18-hr incubation was only slightly greater than the level of inhibition observed at the 4-hr interval for all of the analogs tested with the exception of di-Me-PGA_2 (Fig. 2). In general, di-Me-PGA_1, di-Me-PGA_2, and di-Me-PGE_1 were equally effective in inhibiting $B16_a$ DNA synthesis and were considerably more potent than di-Me-PGE_2 in the 10–25 $\mu g\ ml^{-1}$ range (Fig. 2). As with 3LL tumor cells, PGA and PGE analogs did not alter [^{3}H]thymidine uptake into $B16_a$ cells. Assay of the incubation media for lactate dehydrogenase (11) indicated no difference between control and PG-treated cells. Furthermore, there was no difference in viability (vital dye exclu-

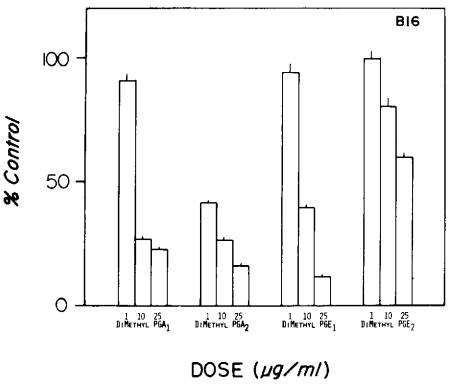

FIG. 2. Dose-dependent inhibition of $B16_a$ tumor cell DNA synthesis by di-Me-PG analogs after 18 hr incubation. See Table II for experimental details.

sion) between control and PG-treated cells, thus excluding the possibility of a cytotoxic effect during the exposure interval studied.

To determine the lag phase between the time of analog administration and the time when they were effective as inhibitors of DNA synthesis, $B16_a$ cells (5×10^5 cells ml^{-1}) were exposed to di-Me-PGA_2 (10 μg ml^{-1}) or di-Me-PGE_2 (10 μg ml^{-1}) in the presence of [^{3}H]thymidine (2 μCi ml^{-1}) for specified times. As seen in Table III, both di-Me-PGA_2 and di-Me-PGE_2 significantly depress DNA synthesis at 30 min postexposure. Inhibition increases with time and at 4 hr di-Me-PGA_2 (10 μg ml^{-1}) depressed $B16_a$ melanoma DNA synthesis 70% below controls. This effect was significantly ($P < 0.005$) greater than the effect of di-Me-PGE_2 at this time (Table II).

The effects of combined treatment with di-Me-PGA_1 and di-Me-PGE_1 on $B16_a$ cellular DNA synthesis were tested (Fig. 3). A combined dose of 2 μg ml^{-1} of each analog (di-Me-PGA_1 + di-Me-PGE_1) inhibited DNA synthesis more than 4 μg ml^{-1} of either di-Me-PGA_1 or di-Me-PGE_1 alone (12 and 28% greater, respectively). Five micrograms per milliliter of each analog inhibited DNA synthesis more than 10 μg ml^{-1} of either di-Me-PGA_1 or di-Me-PGE_1 (17.5 and 23% greater, respectively).

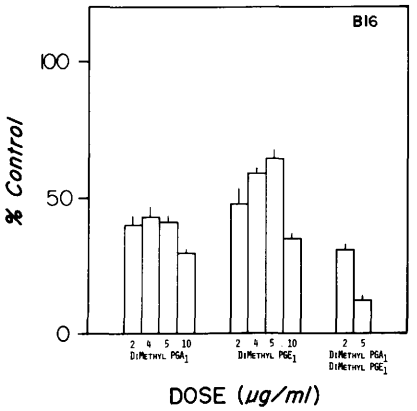

FIG. 3. Synergistic inhibition of $B16_a$ tumor cell DNA synthesis by combination treatment with di-Me-PGA_1 and di-Me-PGE_1. Specific activity of [^{3}H]thymidine = 57.0 Ci $mmole^{-1}$; $n = 4$.

Discussion. PG's and their analogs have been implicated in the control of growth and differentiation of several types of tumor cells (6–8). The majority of published work deals with E series PG's. The analog, di-Me-PGE_2, is a potent inhibitor of tumor growth *in vivo* (7, 12) and induces differentiation of Friend erythroleukemia cells (8). PGA_1 and PGA_2 have been shown to inhibit tumor growth or macromolecular synthesis in a variety of unrelated tumor systems (9, 13–16). PGA_1 is a more potent inducer of Friend erythroleukemia cell differentiation than di-Me-PGE_2 (17). The effects of PGA_1 and PGA_2 on inhibition of Harding–Passey melanoma cell DNA synthesis compare favorably with known chemotherapeutic agents and it has been suggested that these compounds may be an important pharmacological tool in cancer chemotherapy (9).

The di-Me-PGA analogs tested in this report were more effective than di-Me-PGE_2 for inhibition of 3LL tumor cell DNA synthesis and considerably more potent when compared in the $B16_a$ tumor cell system.

TABLE III. TEMPORAL EFFECTS OF DIMETHYL PGA_2 AND DIMETHYL PGE_2 ON DNA SYNTHESIS IN $B16_a$ CELLS

	Time (min)				
	15	30	60	120	180
Dimethyl PGA_2	0.20 ± 0.004[a]	0.33 ± 0.02*	0.47 ± 0.01*	0.94 ± 0.02**	1.22 ± 0.008*
Dimethyl PGE_2	0.18 ± 0.002	0.30 ± 0.002**	0.48 ± 0.01*	0.94 ± 0.02**	1.26 ± 0.02*
Control	0.19 ± 0.005	0.37 ± 0.008	0.53 ± 0.04	1.10 ± 0.02	1.47 ± 0.04

[a] Data are presented as picomoles of thymidine ± SEM per 10^6 cells; $n = 4$ for di-Me-PGA_2 and di-Me-PGE_2 (10 μg ml^{-1}) and $n = 8$ for control. Specific activity of [^{3}H]thymidine; 80 Ci $mmole^{-1}$. Cells (5×10^5 ml^{-1}) were exposed to 2 μCi ml^{-1} [^{3}H]thymidine for the above time intervals.

* Significantly different from control $P \leq 0.05$.

** Significantly different from control $P \leq 0.005$.

Di-Me-PGE_1 is also more effective than di-Me-PGE_2 in both systems. This may be due to the fact that di-Me-PGE_1 is 10 times more effective in increasing cAMP levels in these tumor cells than is di-Me-PGE_2 (unpublished observation). Di-Me-PGA_1 and di-Me-PGA_2 inhibition of DNA synthesis is as effective on a molar basis as retinoic acid inhibition of B16 melanoma cell proliferation (18) and 25 times more effective when compared to retinoic acid effects upon 3LL tumor cell proliferation (19). The effectiveness of the PGA analogs is considerably greater than that of retinyl acetate (18). Retinoic acid inhibition of B16 melanoma cell proliferation requires a 48-hr exposure to the test compound (18) whereas, an equivalent dose of di-Me-PGA_2 significantly inhibits $B16_a$ melanoma cell DNA synthesis within 30 min and an inhibition of 60% was observed within 4 hr. The A and E series PG's and their analogs inhibit tumor growth and DNA synthesis. In addition, they offer the further advantage of inducing tumor cell differentiation (8, 17). Finally, di-Me-PGA and E series compounds can be used in combination therapy to produce a synergistic response.

The mechanism of action of these PG's is unclear, however, it does not appear related to cytotoxicity. *In vitro* PGE compounds are considerably more potent than the PGA compounds in stimulating tumor cell cAMP levels (unpublished observation), suggesting a potentially different mechanism of action for the PGA compounds. Examination of the molecular structure of PGA_1, PGA_2, and their di-Me analogs may provide a clue as to their mechanisms of action. Unlike the other primary prostaglandins (PGE, PGF, PGD, PGC, PGB), only the A series PG's possess a reactive α, β-unsaturated carbonyl group in their cyclopentane ring (20). Various α,β-unsaturated aldehydes have been demonstrated to possess antitumor effects, specifically inhibition of DNA synthesis (21). The B series PG's (PGB_1, PGB_2) are identical in structure to the PGAs, except that they lack a reactive α,β-unsaturated carbonyl group in the cyclopentane ring due to a double-bond shift from carbons 10–11 to carbons 8–12. In contrast to the PGAs, PGB_1 and PGB_2 are poor inhibitors of $B16_a$ and 3LL tumor cell DNA synthesis (unpublished observation).

In vivo, PGA_1 inhibits B16 melanoma tumor growth while stimulating both the humoral and cellular components of the immune response in tumor-bearing immune-suppressed animals (16). The latter effect of the A series PG's may be a factor in their inhibition of tumor growth. However, PGA_1, PGA_2 (9), and their dimethyl analogs have, in addition, potent direct effects on tumor cell DNA synthesis. Collectively, these results suggest their potential efficacy as cancer chemotherapeutic agents.

We thank Dr. J. Pike (Upjohn Company) for providing the prostaglandin analogs used in this study, and Dr. T. T. Tchen for his advice and encouragement during this study. In addition, we thank Dr. B. F. Sloane for performing the lactate dehydrogenase assays. Finally, we thank Dr. M. G. Santoro for her helpful discussions.

1. Vig, B. K., Cancer Chemother. Pharmacol. 3, 143 (1979).
2. Lotan, R., and Dennert, G., Cancer Res. 39, 55 (1979).
3. Lotan, R., and Nicolson, G. L., Cancer Res. 39, 4767 (1979).
4. Christ, E. J., and Van Dorp, D. A., Biochim. Biophys. Acta 270, 537 (1972).
5. Thomas, D. R., Philpott, G. W., and Jaffe, B. M., Exp. Cell Res. 84, 40 (1974).
6. Karim, S. M., Rao, B., and Rao, M., in "Prostaglandins: Physiological, Pharmacological and Pathological Aspects" (S. M. M. Karim, ed.), p. 303. Univ. Park Press, Baltimore (1976).
7. Santoro, M. G., Philpott, G. W., and Jaffe, B. M., Cancer Res. 37, 3774 (1977).
8. Santoro, M. G., Benedetto, A., and Jaffe, B. M., Brit. J. Cancer 39, 259 (1979).
9. Honn, K. V., Dunn, J. R., Morgan, L. R., Bienkowski, M., and Marnett, L. J., Biochem. Biophys. Res. Commun. 87, 795 (1979).
10. Spreafico, F., and Gorattini, S., in "Secondary Spread of Cancer" (R. W. Baldwin, ed.), p. 101. Academic Press, New York (1978).
11. Yochim, J. M., and Clark, F. W., Biol. Reprod. 5, 146 (1971).
12. Tutton, P. J. M., and Barkla, D. H., Brit. J. Cancer 41, 47 (1980).
13. Stein-Werblowsky, R., Experientia 30, 957 (1974).
14. Eisenbarth, G. S., Wellman, D. K., and Lebovitz, H. E., Biochem. Biophys. Res. Commun. 60, 1302 (1974).

15. Honn, K. V., Dawidowicz, K., Bienkowski, M., Morgan, L. R., and Marnett, L. J., Abstr. 4th Intl. Prostaglandin Conf., p. 50. Washington, D.C. (1979).
16. Favalli, C., Garaci, E., Santoro, M. G., Santucci, L., and Jaffe, G. M., Prostaglandins **19**, 589 (1980).
17. Santoro, M. G., Benedetto, A., and Jaffe, B. M., Prostaglandins **17**, 719 (1979).
18. Lotan, R., Giotta, G., Nork, E., and Nicolson, G. L., J. Nat. Cancer Inst. **60**, 1035 (1978).
19. Wilkoff, L. J., Dulmadge, E. A., and Chopra, D. P., Proc. Soc. Exp. Biol. Med. **163**, 233 (1980).
20. Crabbe, P. *In* "Organic Chemistry: Prostaglandin Research" (P. Crabbe, ed.), Vol. 36, p. 1. Academic Press, New York (1977).
21. Schauenstein, E., Esterbauer, H., and Zollner, H., "Aldehydes in Biological Systems" Pion Ltd., London (1977).

Received September 16, 1980. P.S.E.B.M. 1981, Vol. 166.