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INHIBITION OF DNA SYNTHESIS IN HARDING-PASSEY MELANOMA CELLS BY PROSTAGLANDINS A_1 AND A_2: COMPARISON WITH CHEMOTHERAPEUTIC AGENTS*

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SUMMARY: PGA_1 and PGA_2 significantly depressed melanoma cell DNA synthesis and cell proliferation in a dose related fashion. Inhibition of DNA synthesis was rapid in onset (0.5-1 hr) and sustained (12 hr). This was not due to general cytotoxicity or depression of substrate uptake. Comparison with known cancer chemotherapeutic agent revealed PGA_1 and PGA_2 effectiveness on a molar basis exceeded that of Adriamycin, cyclophosphamide and hydroxyurea. Actinomycin D, Mutamycin and 5-fluorouracil were more potent than PGA_1 and PGA_2 but consideration of their toxicities may outweigh this point. The findings suggest that the A series prostaglandins or their analogs may be efficacious in cancer chemotherapy.

Prostaglandin (PG) synthesis and release are increased in a number of tumor types and transformed cells(1). At present it is not known if these elevated tumor PG levels are the result of the transformation to the neoplastic state or whether altered PG biosynthesis contributes to this transformation. This point has been further confused by the fact that the various PG's and PG-like compounds (thromboxanes) have not demonstrated consistent effects on cell division. The majority of this work deals with the PGE and PGF series. Reports dealing with the PGA series are limited and contradictory. PGA_1 decreased cell proliferation and DNA synthesis in chick embryo muscle(2), and human diploid fibroblasts(3), but increased thymic lymphocyte cell proliferation(4). In neoplastic tissue, PGA_1 inhibited growth of a rat chondrosarcoma(5). In the present study PGA_1 and PGA_2 effects on DNA synthesis in a murine melanoma were investigated and compared with cancer chemotherapeutic agents. Both PG's demonstrated rapid onset and long duration of inhibition of melanoma cell DNA synthesis at doses which compared favorably with the chemotherapeutic agents.

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MATERIALS AND METHODS: The NIH Harding-Passey murine melanoma was propagated in male BALB/c mice(6), harvested six weeks post-transplantation, diced and cells isolated by sequential collagenase/trypsin digestion. Cell dispersions and subsequent incubations were performed in Eagles Minimal Essential Media (MEM) with Hank's Balanced Salt Solution (pH 7.4). Briefly, die were digested (30 min; 37°C; Dubnoff metabolic shaker, 170 oscillations/min; 95% O_2 + 5% CO_2) with 0.1% collagenase (Sigma Type I). Suspended cells were collected and the die further treated with two consecutive trypsin (0.5%; Worthington TRL) digestions (20 min; 37°C). Pooled cells were separated by centrifugation (100xg; 30 min; 4°C) washed 1X with MEM + 0.5% bovine albumin (BSA), suspended in MEM + BSA + 0.1% soy bean trypsin inhibitor (Worthington SBI) and counted in a hemocytometer. Cell concentration was adjusted to 5 x 10^5 cells/ml. Viability was determined by vital dye exclusion(7). The effects of PGA_1 and PGA_2 (Upjohn Co.) upon 3H-thymidine (5μCi/ml; Radiochemical Centre, Amersham, 43 Ci/mmol) uptake and incorporation into melanoma DNA were compared with actinomycin D; AMD (Sigma), Adriamycin; ADR (Adria Labs), cyclophosphamide; CPD (Mead-Johnson), 5-fluoro-uracil; 5-FU (Hoffman-LaRoche), Mutamycin; MMC (Bristol) and hydroxyurea; HOU (Schwarz/Mann) at timed intervals. Incubates were centrifuged (12000xg; 4 min) and the pellets homogenized in cold 6% trichloroacetic acid (TCA) and recentrifuged. The acid soluble portion was used as an estimate of thymidine uptake. The pellets were solublized (NCS; 50°C; 36 hrs) bleached (100 μl 30% H_2O_2) and counted by liquid scintillation spectrometry. The specificity of 3H-thymidine incorporation into DNA was verified by dilution with unlabelled thymidine(8) and digestion of TCA precipitated material(8) with deoxyribonuclease I (Worthington). Data are expressed as moles thymidine/10^6 cells or % control. Eight replicate incubations were used per datum point. Data were analyzed by analysis of variance and Student t test. Differences were accepted as significant when $P<0.05$.

RESULTS: Melanoma cells steadily increased thymidine incorporation into DNA during a 12 hr incubation period (Table 1). Cellular uptake of thymidine increased up to 1 hr and then remained unchanged (Table 1). Simultaneous addition of 3H-thymidine with a 10^3 fold molar excess of unlabelled thymidine reduced labelling of precipitated DNA, 98 ± 0.5 and 97 ± 1.2% at 4 and 8 hr. respectively. DNase digestion of 4 and 8 hr pellets reduced labelling 95 ± 1.0 and 97 ± 2.0% respectively.

PGA_1 and PGA_2 (100 μg/ml) significantly depressed DNA synthesis by 0.5 hr (Table 2). At 1 hr both PG's (0.1-100 μg/ml) depressed DNA synthesis, however, PGA_1 was more effective in this regard (Table 2). PGA_1 and PGA_2 continued to exert this effect until maximum inhibition was achieved at 8 hr for all doses studied except PGA_1 (100 μg/ml) which further reduced (150%) DNA synthesis at 12 hr. A slight recovery from inhibition was observed with all doses of PGA_2 at 12 hr (Table 2). Cellular thymidine uptake was not significantly altered from control values by either PGA_1 or PGA_2 in the dose range of 0.1-10 μg/ml.

TABLE 1

^{3}H-thymidine uptake and incorporation by control Harding-Passey melanoma cells

Time hr	0.25	0.5	1	2	4	8	12
Cellular Uptake fmoles/10^6 cells	10±5	30±7	50±10	60±5	50±10	40±10	60±2
Incorporation into DNA pmoles/10^6 cells	0.02±0.002	0.15±0.004	0.37±0.03	1.36±0.07	2.7±0.2	17.6±2.7	30.4±1.4

TABLE 2

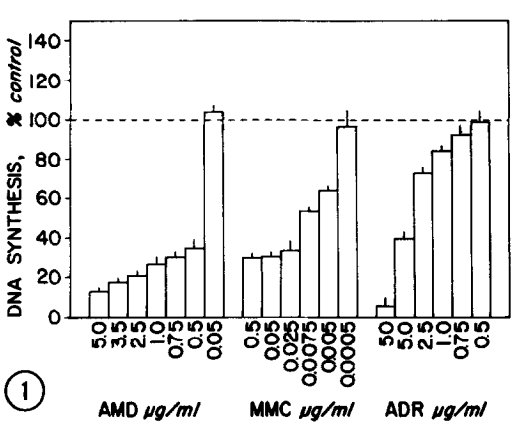
Temporal dose related inhibition (% control) of DNA synthesis by PGA_1 and PGA_2

Time hr.	0.25	0.5	1	2	4	8	12
PGA_1 µg/ml							
0.1	95± 6.0	102±10.0	71± 8.0	73±5.5	60±2.0	53±3.7	49±9.0
1.0	97±10.0	99± 7.0	70±10.0	63±7.2	62±0.7	40±5.0	41±3.0
10	94± 7.0	101± 6.0	85± 2.0	47±3.0	18±0.5	7±0.7	10±0.5
100	106± 5.0	80± 3.0	62± 9.0	49±1.8	12±0.8	5±0.3	2±0.1
PGA_2 µg/ml							
0.1	113±12.0	107± 4.0	90±3.0	88±1.3	70±1.7	61±4.5	69±2.3
1.0	119±6.3	100±5.4	94±2.5	80±2.7	65+3.7	45±0.7	52±1.2
10	96±7.1	101±2.0	86±5.8	56±5.0	26±2.0	15±0.9	23±2.0
100	99±3.5	93±4.7	78±1.3	44±3.2	12±0.4	9±1.0	15±5.0

PGA_1 and PGA_2 (100 µg/ml) reduced cellular uptake of thymidine at 8 hr (17.3 ± 3 and 15 ± 5% respectively).

Cell viability (dye exclusion) of control incubates was unaltered following 8 hr incubation (77 ± 3%, zero time; 79 ± 5%, 8 hr). Neither PGA_1 or PGA_2 (0.1 - 10 µg/ml) significantly decreased cell viability at 8 hr, however, both PG's decreased (PGA_1, 20%; PGA_2, 25%) 8 hr viability at a dose of 100 µg/ml. Cell number increased an average of 63% (8 hr) in control incubates, however, both PG's inhibited this increase over the entire dose range studied. PGA_1 depressed melanoma cell proliferation to a range of 6% (100 µg/ml) to 32% (0.1 µg/ml). Similarly, PGA_2 also depressed cell proliferation; 10% (100 µg/ml), 47% (0.1 µg/ml).

The effects of six chemotherapeutic agents on thymidine uptake and incorporation into DNA were evaluated at 8 hr post treatment and compared to PGA_1



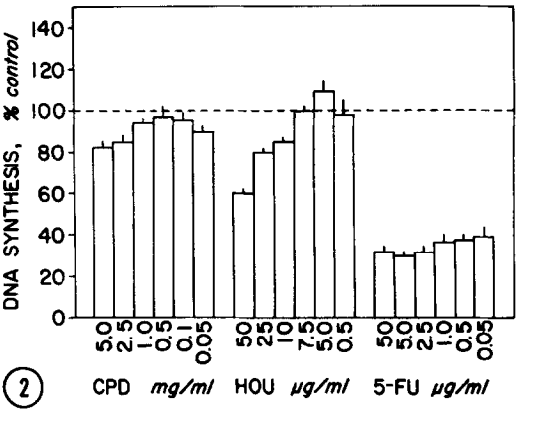
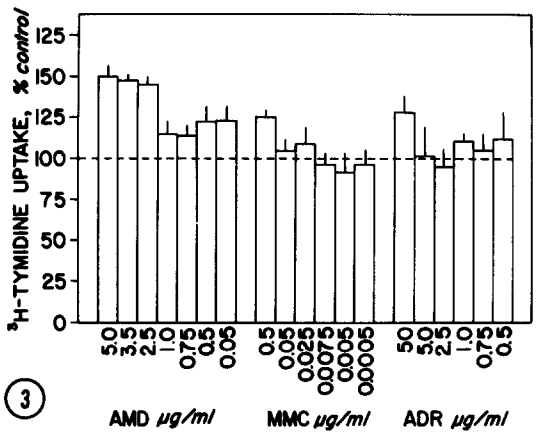


Fig 1. Dose related inhibition of melanoma cell DNA synthesis (8 hr) by actinomycin D (AMD), Mutamycin (MMC) and Adriamycin (ADR)

Fig 2. Effects of cyclophosphamide (CPD), hydroxyurea (HOU) and 5-fluorouracil (5-FU) on melanoma cell DNA synthesis (8 hr)

and PGA_2. AMD and MMC were the most potent DNA synthesis inhibitors with effective dose ranges of 0.5-5 μg/ml and 0.005-0.5 μg/ml respectively (Fig 1). ADR was equipotent at doses approximately 10 and 200 fold greater than AMD and MMC respectively (Fig 1). 5-FU did not demonstrate a dose response over the range (0.5-50 μg/ml) tested and was comparable to AMD and MMC (Fig 2 vs Fig 1). HOU produced a significant reduction of DNA synthesis over the range of 10-50 μg/ml, Whereas CPD was only effective at 5 mg/ml (Fig 2), most likely due to the lack of metabolic activation in this in vitro test system. Relative drug effectiveness for inhibition of melanoma DNA synthesis was MMC>AMD>5-FU>ADR>HOU>>CPD. Melanoma cell thymidine uptake was significantly enhanced by all doses of AMD and 5-FU tested (Figs 3, 4). MMC and ADR significantly increased uptake at 0.5 and 50 μg/ml respectively, all other doses being ineffective (Figs 3, 4). HOU did not alter thymidine uptake from control values, whereas CPD significantly depressed uptake at the higher doses (0.5-5 μg/ml) and stimulated uptake at 0.05 μg/ml (Fig 4). The effectiveness of PGA_1 and PGA_2 on inhibition of DNA synthesis (8 hr) was compared to the chemotherapeutic agents on a molar basis.



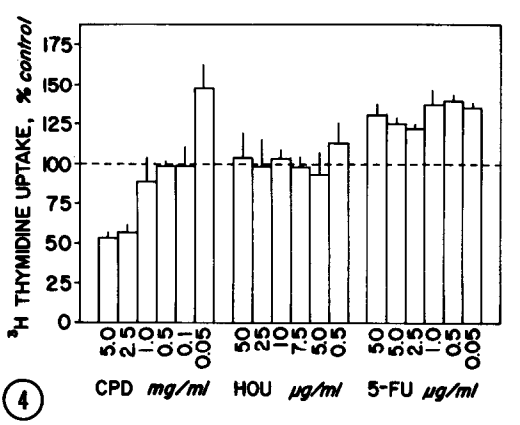


Fig 3. Effect of actinomycin D (AMD), Mutamycin (MMC) and Adriamycin (ADR) on melanoma cell thymidine uptake (8 hr)

Fig 4. Effect of cyclophosphamide (CPD), hydroxyurea (HOU) and 5-fluorouracil (5-FU) on melanoma cell thymidine uptake (8 hr)

The PG's were more effective than ADR (93X), CPD (3533X) and HOU (55X). However, AMD (6.5X) and MMC (14X) were more effective than either PG. The lack of an adequate 5-FU dose response precluded comparison.

DISCUSSION: Exogenous PGE's inhibit DNA synthesis and cell proliferation in leukaemia(9), lymphoma(10) and B-16 murine melanoma cells(11). The synthetic analog of PGE_2, 16, 16-dimethyl PGE_2-methyl ester, when administered *in vivo* delays the appearance of tumors, decreases tumor size and prolongs survival of mice injected with B-16 murine melanoma cells (12). This effect was not due to general cytotoxicity but appeared to be a direct effect on the mitotic process(13). In the present study PGA_1 and PGA_2 depress melanoma cell proliferation and DNA synthesis without altering cell viabilities (except at 100 μg/ml) arguing against a general cytotoxic effect. Furthermore, these effects are not due to PG inhibition of thymidine uptake. In addition the A series PG's are effective at a lower dose (2.78×10^{-7}M) than demonstrated for PGE_1 on the B-16 melanoma(11). In the Harding-Passey melanoma PGA_1 and PGA_2 (10 μg/ml; 4 hr) are 390 and 270% more effective DNA synthesis inhibitors than PGE_1 or PGE_2 respectively (unpublished observation).

The efficacy of PGA_1 and PGA_2 for inhibition of melanoma cell DNA synthesis also compares favorably with known chemotherapeutic agents. Both PG's are considerably more potent than HOU, which is currently used in melanoma chemotherapy(14). Although, AMD, MMC and 5-FU are more potent than the PG's their use is associated with a high degree of bone marrow toxicity whereas PG's have been demonstrated to increase haemopoetic stem cell proliferation(15). Significant inhibition of melanoma cell DNA synthesis and proliferation by A series PG's warrants consideration of their chemotherapeutic potential.

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