CLIN. EXPL. METASTASIS, 1983, VOL. 1, NO. 2, 103–114

Inhibition of tumor cell metastasis by modulation of the vascular prostacyclin/thromboxane A_2 system

KENNETH V. HONN

Departments of Radiation Oncology, Radiology and Biological Sciences, Wayne State University, Detroit, MI 48202, U.S.A.

(Received March 1982; accepted May 1982)

The interaction between metastasizing tumor cells and the hemostatic system of the host has been implicated in successful tumor cell dissemination. Prostacyclin (PGI_2) decreases metastasis from tail vein injected B16 amelanotic melanoma (B16a) cells when administered 15 min prior to tumor cells. This effect is potentiated by a phosphodiesterase inhibitor. Initial trapping of ^{125}I Udr labelled tumor cells in pulmonary vascular beds is unaltered by PGI_2 but retention time is decreased. PGI_2 decreases retention time even when administered 60 min post tumor cells. Structurally unrelated thromboxane (TX) synthetase inhibitors and a TXA_2 receptor antagonist also reduce metastasis from tail vein injected B16a cells. Furthermore, one inhibitor, 1-(7-carboxyheptyl)imidazole, when injected intraperitoneally reduced spontaneous metastasis from subcutaneous B16a and Lewis lung carcinoma tumors. These results suggest that selective manipulation of PGI_2 and TXA_2 can reduce the hematogenous spread of tumor cells.

Patients with malignant neoplasms often demonstrate abnormalities in their blood coagulability [6, 47]. These abnormalities include thrombocytopenia [2], a reduction in fibrinogen and an increase in fibrin–fibrinogen degradation products [6, 23, 47]. Tumor cells have been reported to possess both a platelet activating material [16, 26, 33, 34] and a procoagulant activity responsible for alterations in the fibrin–fibrinogen system [4, 21, 22]. These hemostatic alterations have been linked to tumor cell metastasis which is the process by which tumor cells disseminate from the primary neoplasm to distant organs [6]. Metastasis via the hematogenous route is a complex phenomenon involving detachment of cells from the primary tumor, invasion into blood vessels, transport in the circulatory system, arrest in the microvasculature and finally extravasation into normal tissue followed by growth into a secondary tumor [5]. During their transport in circulation tumor cells are believed to be capable of interacting with host platelets [45]. Several human and animal tumor cells are capable of aggregating platelets *in vitro* [1, 16–18, 30, 32, 34] and manipulation of host platelet levels by induced thrombocytopenia decreases metastatic tumor colony formation in some systems [19, 38]. For those reasons anticoagulant therapy with agents such as heparin [8], warfarin [28] and dipyridamole [20] have been used to reduce tumor cell metastasis with limited success. Recently Honn *et al.* [25] demonstrated that prostacyclin (PGI_2), the most potent antithrombogenic agent known [31] was a potent antimetastatic agent against a murine melanoma. This observation led to the proposed hypothesis [25] that tumor cells can alter the balance between intravascular PGI_2 and platelet generated thromboxane A_2 (TXA_2) in favor of thrombosis. This hypothesis predicted that PGI_2, agents which stimulate or prolong the activity of PGI_2 and thromboxane (TX) synthetase inhibitors would be antimetastatic agents. Experimental evidence is presented to support that hypothesis.

0262 0898 83/0102 0103 $02·00

Materials and methods

Animals and tumors

The B16 amelanotic melanoma (B16a) 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). Routinely, subcutaneous tumors between 1 and 2 g were removed and disaggregated to yield monodispersed cells as described below.

Materials

The TX synthetase inhibitors, 9,11-diazoprosta-5,13-dienoic acid (U51605) and 9,11-iminoepoxyprosta-5,13-dienoic acid (U54701) [14, 12]; a TXA_2 receptor antagonist, 9,11-epoxyiminoprosta-5,13-dienoic acid (U54874) [13]; PGI_2 and 6-keto $PGF_{1\alpha}$ were generously provided by Dr. John Pike, Upjohn Co. U51605, U54701 and U54874 were dissolved in absolute ethanol immediately prior to use. PGI_2 was dissolved in 0·05 M tris buffer pH 9·37 immediately prior to use. All compounds were injected intravenously (tail vein) in a volume of 25 μl. Controls received 25 μl of ethanol or the appropriate buffer. The TX synthetase inhibitor 1-(7-carboxyheptyl)imidazole [46] was generously provided by Ono Pharmaceuticals, Japan. 1-(7-carboxyheptyl)imidazole was dissolved in saline. ^{125}I Udr (specific activity >2000 Ci/mmole) was purchased from New England Nuclear. Collagenase (Type III) was purchased from Worthington Biochemicals. All other reagents were of the highest quality available.

Tumor cell dispersion

Subcutaneous tumors were dispersed as previously reported [39]. Briefly, subcutaneous tumors were removed, diced and placed in sterile Eagle's minimal essential medium (MEM) buffered with sodium bicarbonate (15 mM) and HEPES (25 mM). MEM used for tumor cell dispersion contained collagenase type III (1 mg/ml; 174 U/mg) and fatty-acid-free human serum albumin (Sigma, 10 mg/ml). Cells were dispersed (1 × 30 min and 1 × 60 min, 37°C) under air in a Dubnoff metabolic shaker (90 oscillations/min). Supernatants were collected through cheesecloth, centrifuged (100 *g*, 10 min) and the pellets resuspended in MEM. Viabilities were determined by vital dye exclusion and were generally >93 per cent.

^{125}I Udr labelling

Freshly dispersed tumor cells were adapted for growth in tissue culture medium (MEM, Hank's salts, supplemented with sodium pyruvate, MEM non-essential amino acids, 150 U penicillin-G/ml (Sigma, St. Louis, MO), 100 μg/ml neomycin sulfate (Sigma), 25 mM HEPES (Sigma) and 10 per cent fetal calf serum (FCS) (M.A. Bioproducts, Walkersville, MD)). Cells were allowed to grow to confluency in primary culture, subcultured one time for use in these experiments. For subculturing or enumeration, the cells were detached by a 1 min trypsinization (0·25 per cent trypsin (Worthington, Freehold, NJ), 0·02 per cent EDTA in MEM), pelleted at 100 *g*, 10 min and resuspended in MEM supplemented with FCS, pH 7·3–7·4. The cells were counted in a hemocytometer. Labelling was accomplished by exposing cells to ^{125}I Udr (0·3 μCi/ml) for 18 hours. Cells were harvested as described above, pelleted, resuspended in MEM and washed (twice). Viabilities were generally greater than 97 per cent. Final resuspension for tail vein injection was in MEM. Labelled cells and organs from injected mice were counted in a Searle 1185 dual

channel gamma scintillation spectrometer. Before organs were counted they were extracted in 70 per cent enthanol to remove free ^{125}I as previously described [25].

Lung colony assay

For tail vein injection monodispersed cells were suspended in MEM and injected (50 μl) intravenously into male C57BL/6J mice (10–12 weeks old; 22–27 g). The animals, housed under identical conditions of temperature, photoperiod and feeding, were sacrificed 3 weeks later. Lungs, livers and spleens were removed and fixed in Bouin's solution and the number of metastatic foci in each organ was determined by using a Bausch & Lomb stereo zoom microscope. Kidney and brain were also examined for gross metastatic foci.

Statistics

Data were analyzed by a two-tailed Student's t-test and considered significantly different from controls when $p \leqslant 0{\cdot}05$.

Results

Bolus injection (i.v.) of PGI_2 15 min prior to i.v. injection of 3×10^5 B16a cells resulted in a dose-related decrease in experimental metastasis of the B16a melanoma (figure 1). A significant ($p \leqslant 0{\cdot}05$) decrease was observed with a dose of 25 μg/animal. At the highest dose tested (200 μg) lung metastatic colonies were reduced to 14 per cent of controls. Metastasis to the liver and spleen were also reduced with prostacyclin suggesting that the decrease in lung metastatis does not merely reflect a redistribution of tumor cells (table 1). The byproduct of PGI_2 hydrolysis, 6-keto $PGF_{1\alpha}$ was ineffective at a dose of 50 and 200 μg/animal in reducing pulmonary metastasis (data not shown); however, there was a slight reduction in metastasis to the liver (table 1). Theophylline, a platelet phosphodiesterase inhibitor, at 100 μg/animal (i.p.) produced a slight but insignificant decrease in pulmonary

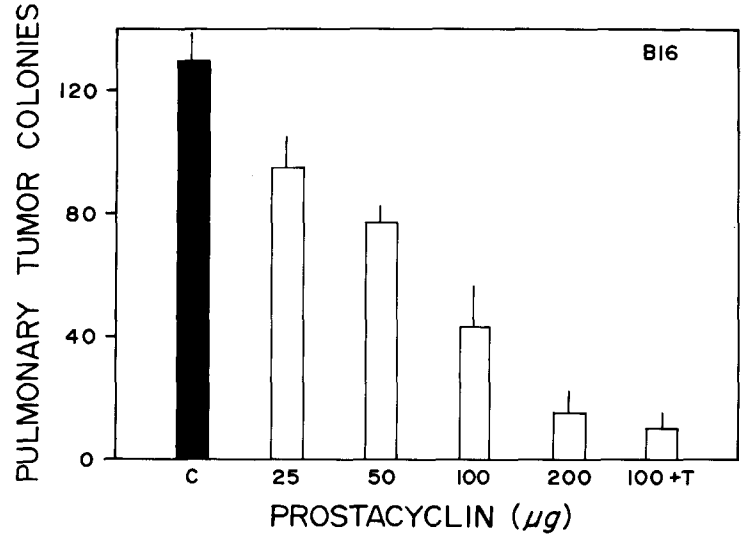

Figure 1. Dose-dependent decrease in metastatic tumor colony formation from i.v. injected B16a cells. PGI_2 was administered i.v. 15 min prior to tumor cell injection. Theophylline (T) was administered (i.p.) 30 min prior to tumor cell injection. Results expressed as mean ± SEM (vertical line), $n = 20$.

Treatment	Liver	Kidney	Spleen	Brain
MEM control	13±5	0	1±0·5	0
TRIS control	8±3	0	3±1	0
PGI_2 25 μg	2±0·5	0	0	0
PGI_2 50 μg	0	0	0	0
PGI_2 100 μg	0	0	0	0
PGI_2 200 μg	0	0	0	0
6-keto $PGF_{1\alpha}$ 50 μg	0	0	0	0
6-keto $PGF_{1\alpha}$ 200 μg	3±1	0	0	0

Table 1. Effects of prostacyclin (PGI_2) on extrapulmonary metastasis following injection of 3×10^5 viable B16 amelanotic melanoma cells.

metastasis. However, the combination of theophylline plus PGI_2 (100 μg/animal) resulted in a fourfold reduction in pulmonary metastasis over PGI_2 alone at the same dose (figure 1).

In order to assess the effect of a single prostacyclin injection on a large circulating tumor burden, mice were pretreated with PGI_2 (150 μg; i.v.) + theophylline (100 μg, i.p.) before the injection of 1×10^6 or 3×10^6 B16a cells. With a tumor burden of 1×10^6 cells, control tumor colonies at 21 days numbered 206±35. PGI_2 + theophylline reduced this number to 9±4 (figure 2). Injection of 3×10^6 cells resulted in more than 500 tumor colonies in the control while PGI_2 + theophylline treated mice exhibited 37±10 colonies (figure 2).

The effects of prostacyclin on the pulmonary retention of B16a cells was evaluated. B16a cells (1×10^6; ^{125}I Udr labelled) were injected (tail vein) into C57BL/6J mice with and without PGI_2 + theophylline pretreatment. All animals had been placed on 0·1 per cent NaI in their drinking water 3 days prior to injection as recommended by Fidler [11]. Lungs were removed at timed intervals. Uptake of labelled cells in control mice lungs was rapid (5 min) with a gradual loss observed after this time until only about 10 per cent of the injected cells remained in the lung at 20 hours (data not shown). Pretreatment with PGI_2 + theophylline did not affect initial cell entrapment in the lung but appeared to affect tumor cell retention time as the loss of tumor cells from the lung was greater at the later time intervals in the PGI_2 + theophylline treated animals (figure 3).

Since PGI_2 has been reported to reverse secondary platelet aggregates [31] it is possible that its effectiveness may extend into a critical period after tumor cell arrest but before extravasation. The period until extravasation is variable with tumor type but is generally considered to be on the order of hours instead of minutes [35]. Therefore, we examined the effects of post administration of PGI_2 + theophylline on the retention of ^{125}I Udr labelled B16a cells. Prostacyclin + theophylline was administered either 15 min prior to tumor cell injection (1×10^6) or 1 hour post tumor cell injection. Animals were then sacrificed 3, 8 and 20 hours post tumor cell injection and the lungs removed and counted. It is obvious from the data in figure 4 that prostacyclin was effective whether administered prior to tumor cell injection or 1 hour post tumor cell injection.

Two TX synthetase inhibitors and a TXA_2 receptor antagonist which are endoperoxide analogs were evaluated in the tail vein model. Although differences in potency were evident all three compounds reduced lung colony formation when injected 15 min prior to 3×10^5 B16a cells (table 2).

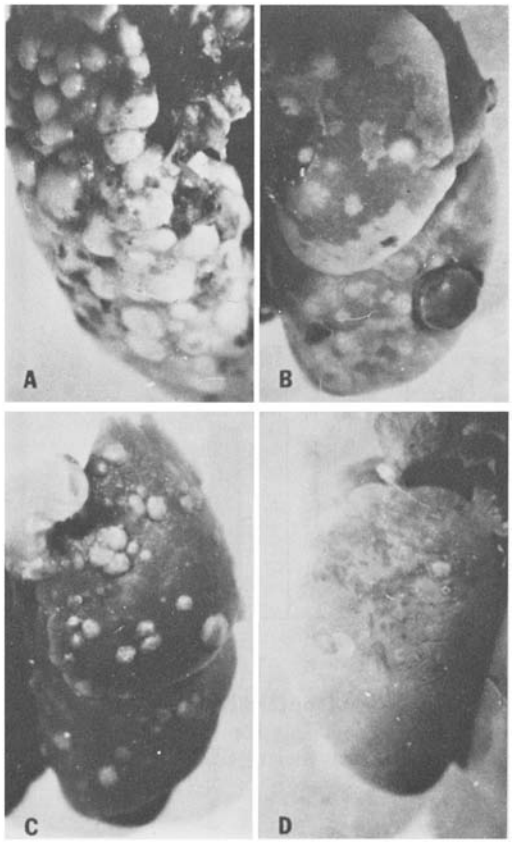

Figure 2. Representative lungs from animals demonstrating the effects of PGI_2 (150 μg, i.v.) + theophylline (100 μg, i.p.) injection 15 and 30 min respectively before injection of a large tumor burden. (A) control; (B) treated, 3×10^6 cells; (C) control; (D) treated, 1×10^6 cells.

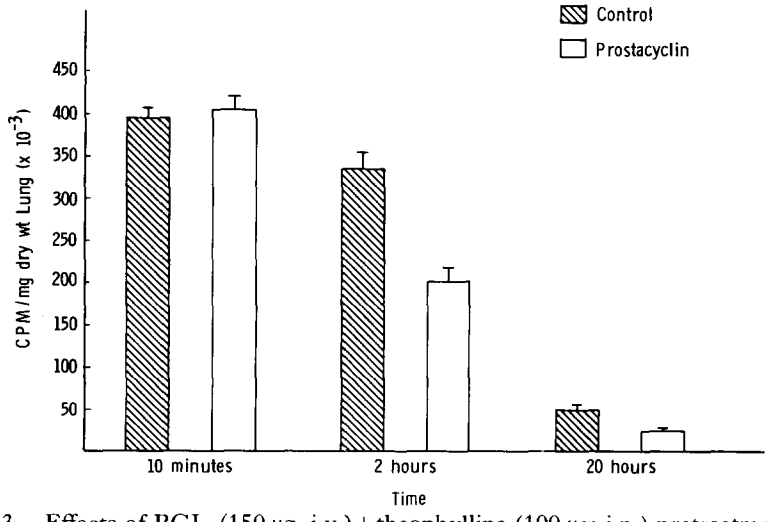

Figure 3. Effects of PGI_2 (150 μg, i.v.) + theophylline (100 μg; i.p.) pretreatment on lung retention of ^{125}I Udr labelled B16a cells. Results expressed as mean $\pm$ SEM, (vertical line), $n = 10$. Time intervals are post tumor cell injection.

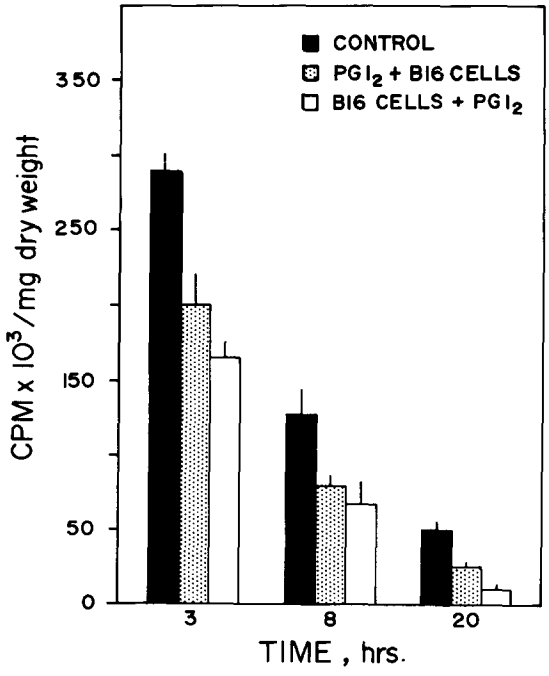

Figure 4. Effects of pretreatment (stippled bar) and post-treatment (white bar) with PGI_2 (150 μg, i.v.) + theophylline (100 μg, i. p.) on retention of ^{125}I Udr labelled B16a cells (1×10^6). Results expressed as mean ± SEM (vertical line), $n = 10$. Time intervals are post tumor cell injection.

Treatment	Tumor colonies
Control[a]	102 ± 26[b]
9,11-diazoprosta-5,13-dienoic acid	
100 μg	75 ± 23
200 μg	56 ± 13*
9,11-iminoepoxy-prosta-5,13-dienoic acid	
100 μg	21 ± 7*
200 μg	6 ± 1·3*
9,11-epoxyimino-prosta-5,13-dienoic acid	
100 μg	47 ± 17*
200 μg	35 ± 9*

[a] 3×10^5 B16a cells injected in 50 μl. [b] Number of metastatic colonies on lung surface (bilateral), mean ± SEM, $n = 10$. *p at least $\leqslant 0{\cdot}05$.

Table 2. Effect of thromboxane synthetase inhibitors and a receptor antagonist on pulmonary metastasis of B16a cells.

In order to determine whether the above effects were peculiar to the endoperoxide structure of the TX synthetase inhibitors we examined the effects of a structurally unrelated TX synthetase inhibitor 1-(7-carboxyheptyl)imidazole (OKY 1553). When injected i.v. 15 min prior to the injection of 1×10^6 B16a cells OKY 1553 produced a dose-dependent decrease in lung colony formation (table 3).

Treatment	Tumor colonies
Control[a]	346±48[b]
OKY 1553/animal	
400 μg[c]	229± 9*
800 μg	118±20*
1600 μg	82±15*
800 μg[d]	194±25*
800 μg[e]	247±45

[a]1×10^6 B16a cells injected in 50 μl. [b]Number of metastatic colonies on lung surface (bilateral), mean±SEM, $n=7$. [c]Administered i.v. 15 min prior to tumour cell injection. [d]Administered i.v. 60 min prior to tumor cell injection. [e]Administered i.v. 60 min post tumor cell injection. *p at least $\leqslant 0{\cdot}05$.

Table 3. Effects of the TX synthetase inhibitor 1-(7-carboxyheptyl)imidazole (OKY 1553) on pulmonary metastasis of B16a cells.

The compound was also effective when administered 1 hour prior to tumor cell injection at a dose of 800 μg/animal, however, a loss of potency was observed when compared to injection 15 min prior to the tumor cells (table 3). OKY 1553 was administered 60 min post tumor cell injection but did not significantly reduce tumor cell metastasis (table 3).

Considering the complexity of events leading to the eventual establishment and growth of a secondary metastatic nodule, there is little doubt that experimental metastasis via tail vein injection of dissociated tumor cells is an artificial and partial model; however, it has its usefulness when standardization of experimental conditions

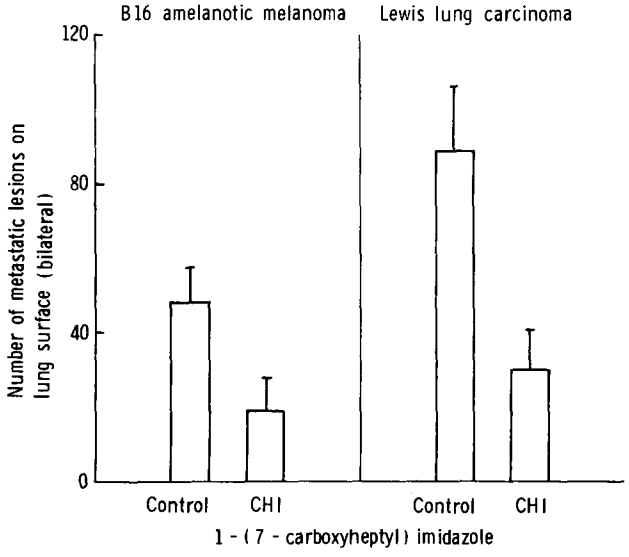

Figure 5. Effects of the TX synthetase inhibitor 1-(7-carboxyheptyl)imidazole on spontaneous metastasis from sc B16a and 3LL tumors. See text for experimental details. Results expressed as mean±SEM (vertical line), $n=20$.

is critical. Nevertheless, we examined the effects of the TX synthetase inhibitor OKY 1553 on spontaneous metastasis from B16a and 3LL tumors. Animals were injected s.c. with 1×10^6 B16a or 3LL tumor cells. Treatment with OKY 1553 (2 mg/animal/day; i.p.) began with the appearance of a palpable tumor (days 5–8 post tumor cell injection). Animals were sacrificed after 26 days and spontaneous metastasis to the lungs determined. A significant decrease in metastasis (60 per cent decrease B16a; 77 per cent decrease 3LL) was observed in both tumor models (figure 5).

Discussion

Prostaglandin research in the past several years has uncovered the critical role played by TXA_2 (formed by platelets) and PGI_2 (formed by vascular endothelium) in the platelet aggregation mechanism [40]. Although it has recently been suggested that three pathways exist for platelet aggregation (i.e., ADP, TXA_2 and 1-*O*-alkyl-2-*O*-acetyl-2sn-glyceryl-3-phosphorylcholine, or platelet activating factor) each can be inhibited by PGI_2 [41]. Therefore, a balance exists between PGI_2 and these aggregating mechanisms, a balance which is necessary for normal hemostasis.

We proposed the hypothesis [25] that the primary tumor, tumor cell shed vesicles [7] and/or circulating tumor cells disrupt this balance in favor of platelet aggregation. The failure and/or divergent results which have been obtained by others attempting anticoagulant therapy to alter metastasis could be due to the fact that the agents tested, with the exception of aspirin, do not impinge directly upon the intravascular PGI_2/TXA_2 balance. If this hypothesis is correct then the following criteria should be substantiated by experimental fact: (1) the exogenous administration of PGI_2 should reduce lung colony formation by tail vein injected tumor cells, (2) a therapeutic synergism should result from the use of PGI_2 with a phosphodiesterase inhibitor, (since the effect of PGI_2 is mediated by increasing concentrations of adenosine 3′,5′-monophosphate (cAMP) in platelets it follows that phosphodiesterase inhibitors by slowing the breakdown of cAMP should potentiate the antithrombotic action of PGI_2 and thus the antimetastatic effect), (3) an inhibitor of endogenous PGI_2 synthesis should enhance metastasis, (4) agents that augment *in vivo* PGI_2 synthesis or activity should function as antimetastatic agents, and (5) thromboxane synthetase inhibitors should also function as antimetastatic agents.

We have demonstrated [25, this study] that PGI_2 possesses significant antimetastatic properties which can be enhanced by a phosphodiesterase inhibitor. In addition, endogenous PGI_2 synthesis may be a natural deterrent to tumor cell metastasis as we have demonstrated that the *in vivo* inhibition of PGI_2 synthetase with hydroperoxy fatty acids [36, 44] significantly increases metastatic tumor colony formation from i.v. injected B16a cells [25].

The exact mechanism for the antimetastatic effects of PGI_2 remains unresolved. It is tempting to speculate and propose that the effect is due to PGI_2 inhibition of tumor cell induced platelet aggregation and platelet tumor cell adhesion. Nevertheless, despite a considerable amount of evidence supporting tumor cell–platelet interaction, the mechanism by which this interaction enhances metastasis awaits rigorous definition. Fantone *et al.* [10] have proposed that PGI_2 decreases adhesiveness of tumor cells stimulated with chemotactic agents (e.g. *N*-formyl-methionyl-leucyl-phenylalanine) to plastic dishes and bovine corneal endothelium. Such decreased adhesiveness could reduce tumor cell-vascular endothelial cell

interaction. It has been observed that tumor cells preferentially attach to damaged endothelium [43, 27]. These interesting results await further clarification; however, pretreatment of B16a tumor cells with PGI_2 prior to tail vein injection does not alter their metastatic behavior [24] and PGI_2 does not affect the adhesiveness of B16a cells in the absence of stimulation by chemotactic agents (unpublished observation).

Finally, we stated that agents which stimulated *in vivo* PGI_2 production should be antimetastatic. Nafazatrom (Bay g 6575) has been reported to possess significant antithrombotic activity in model systems of experimental thrombosis [37]. Thrombus formation in the femoral arteries of rabbits was inhibited at a minimal effective dose of 1 mg/kg p.o. Nafazatrom also possesses significant thrombolytic properties similar to urokinase [42]. The mechanism of action for these antithrombotic effects appears related to the ability of the drug to stimulate PGI_2 production by the vascular wall [42]. Nafazatrom significantly increased bioassayable PGI_2 release from aortic rings obtained from normal and diabetic rats [3]. In addition, plasma, obtained from human volunteers after ingestion of a single dose (1·2 g) of nafazatrom, stimulated PGI_2 release from slices of rat aorta [42]. Nafazatrom also stimulates the biosynthesis of PGI_2 from arachidonic acid by ram seminal vesicle microsomes [9].

We tested the effects of nafazatrom on tail vein and spontaneous metastasis in the B16a and 3LL tumor models and found this compound to be an effective antimetastatic agent [24]. These results lend further support to the contention that PGI_2 is antimetastatic.

It has been demonstrated that tumor cell induced platelet aggregation *in vitro* is accompanied by a parallel stimulation of TXA_2 production [30 and unpublished]. Further, this *in vitro* stimulation of platelet TXA_2 and the accompanying platelet aggregation can be completely inhibited by the TX synthetase inhibitor U54701 (unpublished observation). These *in vitro* results suggest that TX synthetase inhibitors may be antimetastatic. Indeed, that has been demonstrated for structurally unrelated compounds in a melanoma and a carcinoma (this study). Maat and Hilgard [29] have suggested that a lack of correlation exists between the effects of antimetastatic drugs tested in the lung colony assay (tail vein injection) and spontaneous metastasis models with the exception of coumarin anticoagulants such as warfarin. We have demonstrated that a TX synthetase inhibitor (OKY 1553) inhibits metastasis in both models. It has been reported that warfarin has as one of its actions the ability to decrease the half-life of TXA_2, presumably by interfering with its binding to albumin [15]. The relevance of this observation to the antimetastatic effects of warfarin is only conjectural at this time.

In summary, the results presented in this paper demonstrate that *selective* manipulation of the arachidonic acid cascade might be used to therapeutic advantage in control of the hematogenous dissemination of tumor cells.

Acknowledgment

The author thanks Drs. T. T. Tchen and J. D. Taylor for advice and encouragement, and the Department of Chemistry for use of laboratory facilities. Special thanks to M. Patricia Hensler for her expert typing of this manuscript. This work was supported by research grants from the National Institutes of Health (CA29405, CA 29997), the American Cancer Society (BC-356), the Milheim Foundation for Cancer Research and Comprehensive Cancer Center of Metropolitan Detroit.

References

[1] BASTIDA, E., ORDINAS, A., and JAMIESON, G. A., 1981, Idiosyncratic platelet responses to human tumour cells. *Nature, (London)*, **291,** 661–662.

[2] BRAIN, M. C., AZZOPARDI, J. G., BAKER, L. R. I., PINCO, G. F., ROBERTS, P. D., and DACIC, J. V., 1970, Microangiopathic haemolytic anemia: the possible role of vascular lesion in pathogenesis. *British Journal of Haematology*, **18,** 183–190.

[3] CARRERAS, L. O., CHAMONE, D. A. F., KLERCKY, P., and VERMYLEN, J., 1980, Decreased vascular prostacyclin (PGI_2) in diabetic rats. Stimulation of PGI_2 release in normal and diabetic rats by the antithrombotic compound Bay g 6575. *Thrombosis Research*, **19,** 663–671.

[4] CURATOLO, L., COLUCCI, M., CAMBINI, A. L., POGGI, A., MORASCA, L., DONATI, M. B., and SERJERARO, N., 1979, Evidence that cells from experimental tumours can activate coagulation factor X. *British Journal of Cancer*, **40,** 228–233.

[5] DONATI, M. B., DAVIDSON, J. F., and CARATTINI, S., 1981, *Malignancy and the Hemostatic System* (New York: Raven Press), pp. 138.

[6] DONATI, M. B., and POGGI, A., 1980, Malignancy and haemostasis. *British Journal of Haematology*, **44,** 173–182.

[7] DVORAK, H. F., QUAY, S. C., ORENSTEIN, N. R., DVORAK, A. M., HAHN, P., and BITZER, A. M., 1981, Tumor shedding and coagulation. *Science*, **212,** 923–924.

[8] ELIAS, E. G., SEPULVEDA, F., and MINK, I. B., 1973, Increasing the efficiency of cancer chemotherpy with heparin: Clinical study. *Journal of Surgical Oncology*, **5,** 189–193.

[9] ELING, T. E., HONN, K. V., BUSSE, W. D., SEUTER, F., and MARNETT, L. J., 1982, Stimulation of PGI_2 biosynthesis by nafazatrom (Bay g 6575). *Prostaglandins and Cancer*, edited by T. J. Powles, R. S. Bockman, K. V. Honn and P. W. Ramwell (New York: Alan Liss, Inc.), pp. 783–788.

[10] FANTONE, J., KUNKEL, S., and VARANI, J., 1982, Inhibition of tumor cell adherence by prostaglandins. *Prostaglandins and Cancer*, edited by T. J. Powles, R. S. Bockman, K. V. Honn and P. W. Ramwell (New York: Alan Liss, Inc.), pp. 673–678.

[11] FIDLER, I. J., 1978, General considerations for the studies of experimental cancer metastases. *Methods of Cancer Research*, **25,** 399–439.

[12] FITZPATRICK, F. A., BUNDY, G. L., GORMAN, R. R., and HONOHAN, R., 1978, 9,11-Epoxyiminoprosta-5,13-dienoic acid is a thromboxane A_2 antagonist in human platelets. *Nature, (London)*, **275,** 765–766.

[13] FITZPATRICK, F. A., BUNDY, G. L., GORMAN, R. R., HONAHAN, R., MCGUIRE, J., and Sun, F., 1979, 9,11-Iminoepoxyprosta-5,13-dienoic acid is a selective thromboxane A_2 synthetase inhibitor. *Biochimica et biophysica acta*, **573,** 238–244.

[14] FITZPATRICK, F. A., and GORMAN, R. R., 1978, A comparison of imidazole and 9,11-azoprosta-5,13-dienoic acid. Two selective thromboxane synthetase inhibitors. *Biochimica et biophysica acta*, **539,** 162–172.

[15] FULCO, G., GRANSTROM, E., and KINDAHL, H., 1977, Albumin stabilizes thromboxane A_2. *FEBS Letters*, **82,** 321–326.

[16] GASIC, G. J., BOETTIGER, D., CATALFAMO, J. L., GASIC, T. B., and STEWART, G. J., 1978, Aggregation of platelets and cell membrane vesiculation by rat cells transformed *in vitro* by Rous Sarcoma virus. *Cancer Research*, **38,** 2950–2955.

[17] GASIC, G. J., GASIC, T. B., GALANTI, N., JOHNSON, T., and MURPHY, S., 1973, Platelet-tumor cell interactions in mice. The role of platelets in the spread of malignant disease. *International Journal of Cancer*, **11,** 704–718.

[18] GASIC, G. J., GASIC, T. B., and JIMENEZ, S. A., 1977, Platelet aggregating material in mouse tumor cells. *Laboratory Investigation*, **36,** 413–419.

[19] GASIC, G. J., GASIC, T. B., and STEWART, C. C., 1968, Antimetastatic effects associated with platelet reduction. *Proceedings of the National Academy of Sciences of the United States of America*, **61,** 46–52.

[20] GASTPAR, H., 1977, Platelet-cancer cell interaction in metastasis formation: a possible therapeutic approach to metastasis prophylaxis. *Journal of Medicine* (Westbury, New York), **8,** 103–114.

[21] GORDON, S. G., and CROSS, B. A., 1981, A factor X-activating cysteine protease from malignant tissue. *Journal of Clinical Investigation*, **67,** 1665–1671.

[22] GORDON, S. G., FRANKS, J. J., and LEWIS, B., 1975, Cancer procoagulant A: A factor X activating procoagulant from malignant tissue. *Thrombosis Research*, **6,** 127–137.

[23] HARKER, L. A., and SLICHTER, S. J., 1972, Platelet and fibrinogen consumption in man. *New England Journal of Medicine*, **287**, 999–1005.

[24] HONN, K. V., 1982, Prostacyclin/thromboxane ratios in tumor growth and metastasis. *Prostaglandins and Cancer*, edited by T. J. Powles, R. S. Bockman, K. V. Honn and P. W. Ramwell (New York: Alan Liss, Inc.), p. 733.

[25] HONN, K. V., CICONE, B., and SKOFF, A., 1981, Prostacyclin: a potent antimetastatic agent. *Science*, **212**, 1270–1272.

[26] KARPATKIN, S., SMERLING, A., and PEARLSTEIN, E., 1980, Plasma requirement for the aggregation of rabbit platelets by an aggregating material derived from SV40-transformed 3T3 fibroblasts. *Journal of Laboratory and Clinical Medicine*, **96**, 994–1001.

[27] KRAMER, R. H., and NICOLSON, G. L., 1979, Interactions of tumor cells with vascular endothelial cell monolayers: a model for metastatic invasion. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 5704–5708.

[28] LIONE, A., and BOSMANN, H. B., 1978, The inhibitory effect of heparin and warfarin treatments on the intravascular survival of B16 melanoma cells in syngeneic C57 mice. *Cell Biology, International Report*, **2**, 81–86.

[29] MAAT, B., and HILGARD, P., 1981, Anticoagulant and experimental metastases—evaluation of antimetastatic effects in different model systems. *Journal of Cancer Research Clinical Oncology*, **101**, 275–283.

[30] MENTER, D., NEAGOS, G., DUNN, J. R., PALAZZO, R., TCHEN, T. T., TAYLOR, J. D., and HONN, K. V., 1982, Tumor cell induced platelet aggregation: inhibition by prostacyclin, thromboxane A_2 and phosphodiesterase inhibitors. *Prostaglandins and Cancer*, edited by T. J. Powles, R. S. Bockman, K. V. Honn and P. W. Ramwell (New York: Alan Liss), p. 809.

[31] MONCADA, S., and VANE, J. R., 1977, The discovery of prostacyclin—a fresh insight into arachidonic acid metabolism. *Biochemical Aspects of Prostaglandin and Thromboxane Research*, edited by N. Kharasch and J. Fried (New York: Academic Press), pp. 155–177.

[32] PEARLSTEIN, E., AMBROGIO, L., GASIC, G., and KARPATKIN, S., 1981, Inhibition of the platelet-aggregating activity of two human adenocarcinomas of the colon and an anaplastic murine tumor with a specific thrombin inhibitor, dansylarginine *N*-(3-ethyl-1,5-pentanediyl) amide. *Cancer Research*, **41**, 4535–4539.

[33] PEARLSTEIN, E., COOPER, L. B., and KARPATKIN, S., 1979, Correlation between spontaneous metastatic potential, platelet-aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derivatives of a rat renal sarcoma cell line. *Journal of Laboratory and Clinical Medicine*, **93**, 332–244.

[34] PEARLSTEIN, E., SALK, P. L., YOGEESWARAN, G., and KARPATKIN, S., 1980, Correlation between spontaneous metastatic potential, platelet-aggregating activity of cell surface extracts, and cell surface sialylation in 10 metastatic-variant derivatives of a rat renal sarcoma cell line. *Proceedings of the National Academy of Science of the United States of America*, **77**, 4336–4339.

[35] ROOS, E., and DINGEMANS, K. S., 1979, Mechanisms of metastasis. *Biochimica et biophysica acta*, **560**, 135–166.

[36] SALMON, J. A., SMITH, D. R., FLOWER, R. J., MONCADA, S., and VANE, J. R., 1978, Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. *Biochimica et biophysica acta*, **523**, 250–262.

[37] SEUTER, F., BUSSE, W. D., MENG, K., HOSMEISTER, F., MOLLER, E., and HORSTMANN, H., 1979, The antithrombotic activity of Bay g 6576. *Arzneim-Forschung/Drug Research*, **29**, 54–59.

[38] SKOLNIK, G., ALPSTEN, M., and IVARSSON, L., 1980, Studies on mechanisms involved in metastatis formation from circulating tumor cells. *Journal of Cancer Research and Clinical Oncology*, **97**, 249–256.

[39] SLOANE, B. F., DUNN, J. R., and HONN, K. V., 1981, Lysosomal cathepsin B: correlation with metastatic potential. *Science*, **212**, 1151–1153.

[40] SMITH, J. B., 1980, The prostanoids in hemostasis and thrombosis. *American Journal of Pathology*, **99**, 743–804.

[41] VARGAFTIG, B. B., CHIGNARD, M., and BENVENISTE, J., 1981, Present concepts on the mechanism of platelet aggregation. *Biochemical Pharmacology*, **30**, 263–271.

[42] VERMYLEN, J., CHAMONE, D. A. F., and VERSTRAETE, M., 1979, Stimulation of prostacyclin release from vessel wall by Bay g 6575. *Lancet*, **1**, 518–520.

[43] WARREN, B. A., and VALES, O., 1972, The adhesion of thromboplastic tumour emboli to vessel walls *in vivo*. *Journal of Experimental Pathology*, **53**, 301–313.

[44] WEISS, S. J., TURK, J., and NEEDLEMAN, P., 1979, A mechanism for the hydroperoxide mediated inactivation of prostacyclin synthetase. *Blood*, **53**, 1191–1196.

[45] WOOD, S., 1971, Mechanisms of establishment of tumor metastases, *Pathobiology Annual*, Volume 1, edited by H. L. Ioachim (London: Butterworths), p. 281.

[46] YOSHIMOTO, T., YAMAMOTO, S., and HAYAISHI, V., 1978, Selective inhibition of prostaglandin endoperoxide thromboxane isomerase by 1-carboxyalkyl-imidazoles. *Prostaglandins*, **16**, 529–540.

[47] ZACHARSKI, L. R., HENDERSON, W. G., RICKLES, F. R., FORMAN, W. B., CORNELL, JR. C. J., FORCIER, R. J., HARROWER, H. W., and JOHNSON, R. O., 1979, Platelets and malignancy: rationale and experimental design for the VA cooperative study of RA-233 in the treatment of cancer. *Cancer*, **44**, 732–741.