Lack of Effect of in Vivo Prostacyclin on the Development of Pulmonary Metastases in Mice following Intravenous Injection of CT26 Colon Carcinoma, Lewis Lung Carcinoma, or B16 Amelanotic Melanoma Cells

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ABSTRACT

Honn et al. [Science (Wash. DC), 212: 1270, 1981] have recently reported a 93% reduction in the development of metastases of B16 amelanotic tumor cells given i.v. following a single dose of prostacyclin (PGI₂) (100 μg) and theophylline (100 μg) 30 min prior to the injection of tumor cells. We have been unable to reduce pulmonary metastases induced by the i.v. injection of CT26 colon adenocarcinoma, Lewis lung carcinoma, or B16 amelanotic melanoma cells with a similar regimen. Thus, PGI₂ and theophylline given prior to injection of tumor cells and 2 hr postinjection had no effect on the number or volume of pulmonary tumor nodules for CT26 cells, using 15 experimental and 14 control animals; Lewis lung cells, using 14 experimental and 13 control animals; or B16 amelanotic cells, using 26 experimental and 12 control animals. The PGI₂ used was shown to be active in vitro, inhibiting tumor-induced platelet aggregation by all three tumors at 10⁻⁹ M; and in vivo by inhibition of Lewis lung-induced thrombocytopenia at 1 hr, using 100 μg PGI₂ prior to the injection of tumor cells.

INTRODUCTION

In 1968, Gasic et al. (8) reported a reduction in tumor metastases following the induction of thrombocytopenia in the host. This observation prompted several early studies on the ability of antiplatelet agents to inhibit metastases with conflicting results (5, 10, 15, 19, 30).

The resurgence of interest in the role of platelets in tumor cell metastases (1, 3, 12, 18, 20, 21, 23, 25, 26, 28), as well as the pivotal role of prostaglandins (endothelial cell PGI₂ versus platelet thromboxane A₂) in the blood vessel-platelet axis, stimulated the recent study of Honn et al. (16) on the effect of in vivo PGI₂ on the prevention of B16 amelanotic melanoma (B16a) metastases in mice. These workers noted a 70% decrease in the number of metastatic foci in the lungs, as well as a total inhibition of foci in other organs, following a single 100-μg dose of PGI₂, given i.v. 10 min prior to the i.v. injection of tumor cells. A 93% reduction in metastases was noted following the addition of a phosphodiesterase inhibitor (theophylline, 100 μg) given i.p., 30 min prior to the PGI₂ injection. In addition, an inhibitor of PGI₂ synthesis, 15-hydroperoxyarachidonic acid, increased the number of metastases by 163%.

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2 The abbreviations used are: PGI₂, prostacyclin; PBS, 0.01 M phosphate-buffered saline, pH 7.4.

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The volume of each nodule was calculated from its diameter, assuming the node to be a sphere. The mean nodule volume for each mouse was calculated by dividing the total pulmonary nodule volume for each group of mice by the number of mice in each group.

### RESULTS

**Effect of in Vitro Incubation of Tumor Cells at 0° on Cell Viability and Metastatic Potential.** Because preliminary studies had suggested a loss of metastatic potential with time, we determined the effect of *in vitro* incubation of tumor cells on metastatic potential. Table 1 (Experiments 1 to 3) depicts losses of metastatic potential for CT26 cells of 47, 92, and 68%, respectively, following 135 to 150 min of incubation in a test tube immersed in an ice bucket for mean number of pulmonary nodules and 63, 95, and 66%, respectively, for total tumor nodule volume. *In vitro* viability, determined by trypan blue exclusion, declined slightly for Experiment 1 but did not decline for Experiments 2 and 3. Similar results were obtained for Lewis lung and B16a tumor cells (Experiments 4 to 6). *In vitro* viability was not affected.

Further experiments were therefore specifically designed to avoid this loss of metastatic potential following *in vitro* incubation of cells. Accordingly, each control and experimental animal was consecutively alternated for each i.v. tumor injection.

**Effect of PGI₂ and Theophylline on CT26 Pulmonary Metastases.** One hundred μg of the theophylline were given i.p. followed by 100 μg of PGI₂ i.v. 20 min later, followed by 50,000 CT26 tumor cells given i.v. 10 min later into female BALB/c mice; theophylline and PGI₂ were again given 2 hr later. This regimen had no effect on the development of pulmonary metastases per lung as well as total tumor volume per lung (Table 2).

**Effect of PGI₂ and Theophylline on Lewis Lung Pulmonary Metastases.** Similarly, an identical regimen of theophylline and PGI₂ also had no effect on the development of pulmonary metastases following the i.v. injection of 175,000 Lewis lung tumor cells into female C57BL/6J mice.

**Effect of PGI₂ and Theophylline on B16a Pulmonary Metastases.** In this experimental protocol, 2 sets of experimental animals were used: one which received 2 injections of PGI₂ and theophylline as with the previous 2 tumors; and one which simply received a single injection of PGI₂ and theophylline, as reported by Honn et al. (16). Again, PGI₂ and theophylline had no effect on the development of pulmonary metastases in male C57BL/6J mice.

**Effect of PGI₂ on In Vitro Platelet Aggregation Induced by CT26, Lewis Lung, and B16a Melanoma Cells.** Chart 1 demonstrates the effect of PGI₂ on platelet aggregation induced by 50,000 CT26, 500,000 Lewis lung, and 250,000 B16a tumor cells. Complete inhibition of aggregation could be achieved with 10⁻⁹ M PGI₂ for CT26 and B16a cells (equivalent to 0.33 ng/ml platelet-rich plasma) and at 10⁻¹¹ M for Lewis lung cells (equivalent to 3.3 ng/ml platelet-rich plasma).

**Effect of PGI₂, Given *In Vivo*, on Lewis Lung-Induced Thrombocytopenia.** Table 3 depicts the reduction of tumor-induced thrombocytopenia by preinjection of mice with theophylne and PGI₂. In Experiments 1, 1.5 × 10⁶ Lewis lung cells given i.v. reduced the platelet count to 59% of the base-line value at 1 hr and 85% of this value at 2 hr. Pretreatment with theophylline plus PGI₂ completely prevented the induction of thrombocytopenia (Experiment 1). Similarly, 4 × 10⁶ Lewis lung cells reduced the platelet count to 28% of base line at 1 hr. Pretreatment with PGI₂ and theophylline partially prevented the induction of thrombocytopenia to 44% of base line 𝜌 < 0.05 (Experiment 2).
DISCUSSION

Although considerable evidence exists for a role for platelets in the development of tumor metastases in both the older (2, 4–9, 13, 14, 17, 19, 27, 29) and recent (1, 3, 10, 12, 18, 20, 21, 23, 25, 26, 28) literature, there is controversy regarding the efficacy of antiplatelet agents in the prevention of metastases. Thus, although Gasic et al. (5) reported beneficial effects of the use of aspirin in mice given injections of MCA2 and T241 fibrosarcoma cells and Kolenich et al. (19) made similar observations on BW10232 adenocarcinoma of rabbits, Wood and Hilgard (30) obtained negative results with a V2 carcinoma of rabbits, and Hilgard et al. (15) obtained negative results in a careful study with Lewis lung carcinoma in mice using various antiplatelet agents: aspirin, bencyclane, and RA 233 (a dipyrindamole derivative). Furthermore, although Gordon et al. (10) obtained positive results with a Wilms' tumor of Wistar-Furth rats, equivocal results were obtained with a neuroblastoma (C1300) of mice, and negative results were obtained with an NIH renal adenocarcinoma of mice, using pentoxifyllin (a phosphodiesterase inhibitor) as an antiplatelet agent.

Therefore, the recent report of Honn et al. (16) on the dramatic reduction of B16a metastases in mice by the use of a combination of PGI2 and theophylline was very encouraging. Our inability to reproduce their results, with the use of 3 different mouse tumors, including the same B16a pedigree line utilized by Honn et al. (16), was disappointing.

Several possibilities should be considered which might explain the discrepant results obtained from both laboratories: (a) the PGI2 which we used was inactive. This was shown not to be the case by our in vitro studies using platelet aggregate and in vivo studies using tumor-induced thrombocytopenia; (b) different tumors were used. One of our tumor cell lines, B16a, was also used by Honn et al. (16); (c) the experimental pharmacological protocol was different. Although we used 2 dosages of PGI2 and theophylline for the 3 tumor cell lines used, an identical pharmacological protocol (single dose of PGI2 and theophylline) was also used with the B16a tumor cell line; (d) method of preparation of the tumor cell lines for injection in vivo. The 3 lines that we used were obtained by trypsin treatment of subconfluent tissue culture dishes, whereas the B16a cell line used by Honn et al. was removed directly from animals bearing the tumor, diced, dispersed, treated with collagenase and DNase, exposed to soybean trypsin inhibitor, and collected through cheesecloth prior to centrifugation and washing of cells. This preparation may have included macrophages which have recently been shown to dramatically increase the number of i.v. administered metastases if injected prior to or immediately after the tumor cell inoculation (11). It is conceivable that PGI2 may have inhibited this macrophage-enhancement effect; (e) Honn et al. (16) injected approximately 10 times as many B16a tumor cells (3 x 105) as were injected in our experiment (2.5 x 104) and obtained approximately 6 times as many control pulmonary nodules as in our experiments. However, one would expect a greater effect for an antimetastatic pharmacological agent(s) if the number of control pulmonary nodules were decreased rather than increased; (f) finally, the manner in which the tumor cells were injected into the control and experimental animals is not stated in the paper of Honn et al. (16). In our experience, considerable systematic error can be introduced if control animals are given injections first as a group, followed by the injection of experimental animals. It is

Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control vehicle + PGI2 + theophylline</th>
<th>Vehicle + tumor cells at 1 hr</th>
<th>Vehicle + tumor cells at 2 hr</th>
<th>PGI2 + theophylline + tumor cells at 1 hr</th>
<th>PGI2 + theophylline + tumor cells at 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.54 ± 0.03 (6)</td>
<td>0.81 ± 0.05 (4)</td>
<td>1.31 (2)</td>
<td>1.51 ± 0.02 (4)</td>
<td>1.55 (2)</td>
</tr>
<tr>
<td>2</td>
<td>1.60 ± 0.06 (5)</td>
<td>0.45 ± 0.05 (4)</td>
<td>1.51 ± 0.02 (4)</td>
<td>0.71 ± 0.08 (4)</td>
<td></td>
</tr>
</tbody>
</table>

* Viable Lewis lung tumor cells (150,000) were injected i.v. into C57BL/6J mice after pretreating the mice with 100 μg of theophylline given i.p. 30 min prior to injection of tumor cells and 100 μg of PGI2 given 20 min prior to injection of tumor cells. Animals were followed for 1 and 2 hr.

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not clear whether this may have contributed to the differences in results obtained by both laboratories.

The proposal that PGI_2 may be an effective antimetastatic agent (16) is reasonable if platelet aggregation plays a role in the development of tumor metastases, since PGI_2 is a potent inhibitor of platelet aggregation at 10^{-9} M and 100 μg of PGI_2 inhibited the in vivo induction of thrombocytopenia in our animals. Unfortunately, PGI_2 plus theophylline, given prior to the injection of tumor cells and 2 hr postinjection, had no effect on pulmonary metastases. If platelet aggregation and release do play a role in the development of tumor metastases, then it is possible that the short half-life of PGI_2 (relative to the duration of the platelet contribution to metastasis) may have contributed to the negative results. In addition, it is possible that some property of platelets that is not affected by PGI_2 may be responsible for the role of platelets in tumor metastases. In the following paper (24), we present evidence that platelets are required for the development of optimum tumor metastases.

REFERENCES


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