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# Cyclooxygenase and lipoxygenase inhibitors as modulators of cancer therapies

**Beverly A. Teicher[1,2], Timothy T. Korbut[1], Krishna Menon[1], Sylvia A. Holden[1], Gulshan Ara[1]**

[1] Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[2] Joint Center for Radiation Therapy, 44 Binney Street, Boston, MA 02115, USA

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**Abstract.** Like many clinical non-small-cell lung cancers, the Lewis lung carcinoma produces prostaglandins. The Lewis lung carcinoma was used as a model of both primary and metastatic disease to assess the ability of cyclooxygenase inhibitors (mefenamic acid, diflunisal, sulindac, and indomethacin), the collagenase inhibitor minocycline, and the lipoxygenase inhibitor phenidone to act as modulators of cytotoxic cancer therapies. Although none of the single modulators given i.p. daily on days 4–18 altered tumor growth or the number of metastases found on day 20, modulator combinations consisting of minocycline/a cyclooxygenase inhibitor and, especially, of phenidone/a cyclooxygenase inhibitor resulted in modest tumor growth delay and a decreased number of lung metastases on day 20. The most effective modulators of cisplatin (CDDP) were phenidone/sulindac and phenidone/indomethacin, which led to 2.4- to 2.5-fold increases in the tumor growth delay produced by CDDP. The most effective modulations of cyclophosphamide resulted from administration of minocycline, minocycline/sulindac, or phenidone/sulindac and led to 2.0- to 2.1-fold increases in tumor growth delay by cyclophosphamide. The most effective modulators of melphalan produced 4.5- to 4.7-fold increases in tumor growth delay by the drug and were minocycline/sulindac, minocycline/mefenamic acid, and phenidone/sulindac. The most effective modulation of carmustine (BCNU) was obtained with minocycline/sulindac and minocycline/diflunisal leading to 2.8- to 3.1-fold increases in tumor growth delay by BCNU. Finally, the most effective modulation of radiation was obtained with minocycline/sulindac and phenidone/sulindac and resulted in 2.8- to 3.3-fold increases in tumor growth delay by radiation. The modulator combination that along with the cytotoxic therapies was most effective against metastatic disease was phenidone/mefenamic acid. There was no clear relationship between effective modulation of the cancer therapies and the degree of reduction in serum levels of prostaglandin $E_2$ and leukotriene $B_4$ by the agents in Lewis lung tumor bearing mice.

## Introduction

Non-small-cell lung cancer is traditionally regarded as a chemotherapy-refractory disease [51]. Numerous combinations of cytotoxic agents have been tried both preclinically and clinically against non-small-cell lung cancer. In cell culture, human non-small-cell lung cancer lines tend to be among the more drug-resistant cell lines developed from other human solid tumor types [18, 60].

Growth in vivo requires that tumor cells restructure the surrounding extracellular matrix, initiate proliferation of critical normal cells such as endothelial cells, and, perhaps, alter host defense systems [31, 38, 61]. Products of arachidonic acid metabolism are involved in intercellular signaling [20, 39, 46, 50, 55]. In a study of 55 human solid tumor cell lines, 15 of the 19 non-small-cell lung carcinoma cell lines secreted prostaglandin $E_2$ and $F_{2\alpha}$ into the cell culture media at relatively high levels, whereas only 6 of the remaining 36 cell lines produced any detectable prostaglandin $E_2$ [32–35]. The lung cancer cell lines that secreted the prostaglandins were bronchioalveolar cell (2 of 2) adenocarcinoma (9 of 10) and large cell undifferentiated carcinoma (3 of 3). Two squamous-cell carcinomas of the lung cell lines did not produce detectable prostaglandin $E_2$ or $F_{2\alpha}$. In a study comparing profiles of endogenous prostaglandin $E_2$ production in matched unstimulated normal lung and lung carcinoma, tissue-prostaglandin $E_2$ levels were higher in all primary lung-tumor histological cell types (squamous-cell, $n = 20$; adenocarcinoma, $n = 7$; small-cell, $n = 4$; mixed-cell, $n = 2$; bronchioalveolar cell,

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*Correspondence to:* Dr. Beverly A. Teicher, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA

$n = 2$; bronchial carcinoid, $n = 1$) with the exception of large cell undifferentiated carcinomas ($n = 3$) [40].

It has been recognized for some time that the murine Lewis lung carcinoma secretes prostaglandin $E_2$ and that administration of the prostaglandin synthesis inhibitor indomethacin beginning at the time of tumor cell implantation can slow the growth and metastasis of the tumor [11, 16, 17, 65–73]. The secretion of prostaglandin $E_2$ by both Lewis lung tumor cells and host macrophages correlated with the suppression of host natural killer cell and T-lymphocyte cytotoxic activity in mice bearing the tumor. The role of prostaglandin secretion in the metastatic potential of Lewis lung tumor cells remains under investigation and may be correlated with the level of lamin receptor expression by these cells [3, 4, 16, 17].

Arachidonic acid is metabolized through three pathways [46, 55]. The first, which leads to the prostanoids (thromboxanes and prostaglandins), is initiated by the enzyme cyclooxygenase (PGG/H synthase). The second, which leads to the leukotrienes, is initiated by the enzyme lipoxygenase, and the third, which leads to the epoxyeicosatrienoic acids, is initiated by cytochrome P-450 epoxygenases. Most of the work examining the relationships between eicosanoids and malignant disease has focused on the prostanoids [1, 2, 5, 9, 31, 33, 37, 40, 63]. The enzyme cyclooxygenase is found in most tissues and intracellularly is found in greatest abundance in the endoplasmic reticulum [8, 30, 55]. Cyclooxygenase metabolizes arachidonic acid to prostaglandin endoperoxide $H_2$, which leads to thromboxane $A_2$, prostacyclin, and various prostaglandins. These molecules are positive and negative effectors of various metabolic processes operating through specific plasma membrane receptors coupled to G proteins that activate intracellular message transduction [31, 46, 55]. Specifically, prostaglandins have been implicated in the control of the production of collagenase IV, thromboxane $A_2$, and prostacyclin, which are positive and negative regulators of platelet aggregation and vasodilation as well as endothelial and, perhaps, malignant cell proliferation [8, 30, 31, 46, 55]. The level of cyclooxygenase protein has been shown to be influenced in various cell and organ systems by steroids, growth factors, cytokines, and tumor promoters, suggesting that regulation of the level of this enzyme is an important part of regulating prostanoid formation.

Numerous inhibitors of cyclooxygenase and lipoxygenase have been prepared. The current study examines the effect of several cyclooxygenase inhibitors as well as the lipoxygenase inhibitor phenidone and the collagenase IV inhibitor minocycline on the efficacy of cytotoxic anticancer therapies against primary and metastatic disease in the Lewis lung carcinoma.

## Materials and methods

*Drugs.* Phenidone, minocycline, mefenamic acid, diflunisal, sulindac, indomethacin, cyclophosphamide, *cis*-diamminedichloroplatinum(II) (CDDP), and melphalan were purchased from Sigma Chemical Co. (St. Louis, Mo.). BCNU (carmustine) was purchased from the Dana-Farber Cancer Institute pharmacy.

*Tumor.* The Lewis lung tumor [52, 56, 57] was carried in male C57BL mice (Taconic Laboratories, Germantown, N.Y.). For the experiments, $2 \times 10^6$ tumor cells prepared from a brei of several stock tumors were implanted s.c. into the legs of male mice aged 8–10 weeks.

*Tumor growth-delay experiments.* By day 4 after tumor cell implantation, Lewis lung tumors have begun neovascularization [27, 28]. Animals bearing Lewis lung tumors were treated daily with i.p. phenidone (50 mg/kg), minocycline (10 mg/kg), mefanimic acid (10 mg/kg), diflunisal (15 mg/kg), sulindac (15 mg/kg), or indomethacin (5 mg/kg) alone or in combination on days 4–18 following tumor implantation. When the tumors had reached a volume of approximately 100 $mm^3$ (day 7 after tumor cell implantation), cytotoxic therapy was initiated. CDDP (10 mg/kg) or melphalan (10 mg/kg) was given i.p. on day 7 after tumor implantation. BCNU (15 mg/kg) was given i.p. on days 7, 9, and 11 following tumor implantation. Radiation was delivered locally to the tumor-bearing limb as 3 Gy given daily on days 7–11.

The progress of each tumor was measured thrice weekly until it had reached a volume of 500 $mm^3$. Tumor growth delay was calculated as the number of days required for each treated tumor to reach a volume of 500 $mm^3$ as compared with untreated control tumors. Each treatment group comprised six animals and the experiment was repeated three times. The doubling time of the Lewis lung carcinoma was 2.5 $\pm$ 0.2 days. Tumor growth-delay data are presented as the mean value $\pm$ SE calculated for the treatment group as compared with the control group [59].

*Lung metastases.* In animals treated as described above, the number of external lung metastases present on day 20 after tumor implantation were counted manually and scored as $\geq$3 mm in diameter. The data shown are the means from 6–12 pairs of lungs. Parentheses indicate the percentage of large (vascularized) metastases [59].

*Radioimmunoassay.* Serum levels of prostaglandin $E_2$ and leukotriene $B_4$ were measured in normal and Lewis lung tumor-bearing mice using radioimmunoassay (kits NEK-020 and NEK-037, Dupont NEN Research Products, Boston, Mass.). Blood from five animals was pooled for each assay. Animals received no treatment or were given daily i.p. injections of the cyclooxygenase inhibitors, phenidone, or minocycline alone or in combination beginning on day 4 following tumor cell implantation. Each assay was performed four independent times.

## Results

Each of the cyclooxygenase inhibitors mefenamic acid, diflunisal, sulindac, and indomethacin, the lipoxygenase inhibitor phenidone, and the collagenase IV inhibitor minocycline were given daily by i.p. injection for 2 weeks beginning on day 4 after s.c. implantation of the Lewis lung carcinoma in the hind legs of male C57BL mice. Untreated control animals bearing this tumor will die from lung metastases within 22–25 days of s.c. tumor implantation. Each of the potential modulators was at a well-tolerated dose (Table 1).

On this 2-week treatment schedule, none of these agents significantly impacted on tumor growth or metastasis. Administration of CDDP (10 mg/kg) resulted in a tumor growth delay of 4.5 days in the Lewis lung carcinoma. Daily administration of phenidone increased the tumor growth delay produced by CDDP by a factor of 1.8, whereas administration of diflunisal or sulindac increased the tumor growth delay produced by CDDP by a factor of 1.3. Cyclophosphamide ($3 \times 150$ mg/kg) was very effective against the Lewis lung tumor, resulting in a tumor growth delay of 21.5 days. The best modulator of cyclophos-

**Table 1.** Growth delay of the Lewis lung carcinoma and numbers of lung metastases on day 20 after s.c. tumor implantation as produced by treatment with single modulators and cytotoxic cancer therapies

| Treatment | Tumor growth delay, days | Number of lung metastases (% large) |
|---|---|---|
| None | | 20 (62) |
| Phenidone (50 mg/kg) | 1.2±0.4 | 21 (44) |
| Minocycline (10 mg/kg) | 0.8±0.3 | 20 (50) |
| Mefenamic acid (10 mg/kg) | 1.0±0.3 | 19 (53) |
| Diflunisal (15 mg/kg) | 0.3±0.3 | 20 (51) |
| Sulindac (15 mg/kg) | 1.2±0.4 | 19 (55) |
| Indomethacin (5 mg/kg) | 1.2±0.4 | 18 (53) |
| CDDP (10 mg/kg) | 4.5±0.3 | 13 (58) |
| Phen/CDDP | 8.0±0.7 (1.8)* | 15 (43) |
| Mino/CDDP | 5.0±0.3 (1.1) | 11 (48) |
| Mefen A/CDDP | 4.8±0.4 (11) | 14 (43) |
| Diflun/CDDP | 5.8±0.5 (1.3) | 15 (37) |
| Sulin/CDDP | 5.9±0.5 (1.3) | 13.5 (44) |
| Indo/CDDP | 3.7±0.4 (0.8) | 13 (40) |
| Cyclophosphamide (3×150 mg/kg) | 21.5±1.7 | 12 (40) |
| Phen/CTX | 17.6±1.8 (0.8) | 11 (35) |
| Mino/CTX | 45.2±2.9 (2.1)* | 6 (33) |
| Mefen A/CTX | 28.4±3.1 (2.1)* | 10 (40) |
| Diflun/CTX | 31.5±2.8 (1.5)* | 8 (38) |
| Sulin/CTX | 35.1±2.6 (1.6)* | 10 (30) |
| Indo/CTX | 22.5±2.8 (1.0) | 9 (30) |
| Melphalan (10 mg/kg) | 2.7±0.3 | 13 (48) |
| Phen/PAM | 5.8±0.6 (2.1)* | 16 (39) |
| Mino/PAM | 4.3±0.3 (1.6)* | 11 (50) |
| Mefen A/PAM | 4.5±0.3 (1.7)* | 13 (30) |
| Diflun/PAM | 5.0±0.3 (1.9)* | 14 (40) |
| Sulin/PAM | 7.2±0.4 (2.7)* | 12 (48) |
| Indo/PAM | 4.4±0.3 (1.6)* | 10 (47) |
| BCNU (3×15 mg/kg) | 3.6±0.4 | 15.5 (53) |
| Phen/BCNU | 4.8±0.5 (1.3) | 12 (39) |
| Mino/BCNU | 5.2±0.6 (1.4) | 15 (38) |
| Mefen A/BCNU | 5.3±0.5 (1.5)* | 10 (57) |
| Diflun/BCNU | 4.7±0.5 (1.3) | 12 (50) |
| Sulin/BCNU | 7.0±0.6 (1.9)* | 12 (30) |
| Indo/BCNU | 6.2±0.8 (1.7)* | 13 (45) |
| X-rays (5×3 Gy) | 4.4±0.3 | 15 (40) |
| Phen/5×3 Gy | 5.6±0.5 (1.3) | 14 (36) |
| Mino/5×3 Gy | 7.8±0.6 (1.8)* | 13 (30) |
| Mefen A/5×3 Gy | 6.7±0.6 (1.5)* | 15 (40) |
| Diflun/5×3 Gy | 6.5±0.5 (1.5)* | 13.5 (44) |
| Sulin/5×3 Gy | 7.3±0.7 (1.7)* | 14 (36) |
| Indo/5×3 Gy | 6.3±0.5 (1.4) | 15 (40) |

Parentheses indicate an x-fold increase in tumor growth delay as compared with that produced by the antitumor alkylating agent alone
* Statistically significant increase in tumor growth delay ($P < 0.01$)

**Table 2.** Growth delay of the Lewis lung carcinoma and numbers of lung metastases on day 20 after s.c. tumor implantation as produced by treatment with minocycline and a cyclooxygenase inhibitor along with cytotoxic cancer therapies

| Treatment | Tumor growth delay, days | Number of lung metastases (% large) |
|---|---|---|
| Minocycline/sulindac | 3.1±0.4 | 16 (38) |
| Minocycline/diflunisal | 1.4±0.4 | 16 (63) |
| Minocycline/indomethacin | 3.0±0.3 | 16 (56) |
| Minocycline/mefenamic acid | 0.9±0.5 | 13 (63) |
| CDDP (10 mg/kg) | 4.5±0.3 | 13 (58) |
| Mino/Sulin/CDDP | 8.6±0.8 (1.9)* | 14 (36) |
| Mino/Diflun/CDDP | 7.6±0.7 (1.7)* | 15 (40) |
| Mino/Indo/CDDP | 5.9±0.6 (1.3) | 14 (43) |
| Mino/Mefen A/CDDP | 6.7±0.6 (1.5)* | 8 (30) |
| Cyclophosphamide (3×150 mg/kg) | 21.5±1.7 | 12 (40) |
| Mino/Sulin/CTX | 43.7±3.6 (2.0)* | 13 (23) |
| Mino/Diflun/CTX | 32.3±3.3 (1.5)* | 12.5 (32) |
| Mino/Indo/CTX | 36.6±2.9 (1.7)* | 13.5 (33) |
| Mino/Mefen A/CTX | 27.9±2.8 (1.3) | 6.5 (44) |
| Melphalan (10 mg/kg) | 2.7±0.3 | 13 (48) |
| Mino/Sulin/PAM | 12.1±1.3 (4.5)** | 7.5 (35) |
| Mino/Diflun/PAM | 10.2±1.2 (3.8)** | 6.5 (38) |
| Mino/Indo/PAM | 6.3±0.5 (2.3)* | 12 (41) |
| Mino/Mefen A/PAM | 12.6±1.2 (4.7)** | 8 (43) |
| BCNU (3×15 mg/kg) | 3.6±0.4 | 15.5 (53) |
| Mino/Sulin/BCNU | 9.9±0.9 (2.8)** | 16 (31) |
| Mino/Diflun/BCNU | 11.2±1.4 (3.1)** | 15.5 (35) |
| Mino/Indo/BCNU | 9.1±0.9 (2.5)** | 10 (30) |
| Mino/Mefen A/BCNU | 7.6±0.7 (2.1)* | 10 (30) |
| X-rays (5×3 Gy) | 4.4±0.3 | 15 (40) |
| Mino/Sulin/5×3 Gy | 12.5±1.2 (2.8)** | 13 (38) |
| Mino/Diflun/5×3 Gy | 9.2±1.1 (2.1)* | 12.5 (40) |
| Mino/Indo/5×3 Gy | 8.8±0.9 (2.0)* | 13 (38) |
| Mino/Mefen A/5×3 Gy | 11.1±0.9 (2.5)** | 13 (38) |

Parentheses indicate an x-fold increase in tumor growth delay as compared with that produced by the antitumor alkylating agent alone
* $P < 0.01$, ** $P < 0.005$

phamide was minocycline, which increased the tumor growth delay produced by cyclophosphamide by a factor of 2.1. Sulindac administration increased the tumor growth delay produced by cyclophosphamide by a factor of 1.6. A single dose of 10 mg/kg melphalan resulted in 2.7 days of tumor growth delay in the Lewis lung tumor. Daily administration of sulindac resulted in a 2.7-fold increase in the tumor growth delay produced by melphalan, whereas daily administration of the lipoxygenase inhibitor phenidone produced a 2.1-fold increase in the tumor growth delay elicited by melphalan. BCNU (3 × 15 mg/kg) administration resulted in 3.6 days of tumor growth delay in the Lewis lung tumor. Daily administration of sulindac increased the tumor growth delay produced by BCNU by a factor of 1.9, whereas daily administration of indomethacin increased the tumor growth delay produced by BCNU by a factor of 1.7. Minocycline was the most effective modulator of fractionated radiation therapy, increasing the tumor growth delay from 4.4 days to 7.8 days, corresponding to a 1.8-fold change. Sulindac administration increased the tumor growth delay resulting from fractionated radiation by a factor of 1.8.

Each of the four cyclooxygenase inhibitors was studied in combination with the collagenase IV inhibitor minocycline (Table 2). The two most effective modulator combinations were minocycline with sulindac and minocycline with indomethacin. The daily administration of minocycline and sulindac increased the tumor growth delay produced by CDDP by a factor of 1.9 and increased the tumor

**Table 3.** Growth delay of the Lewis lung carcinoma and numbers of lung metastases on day 20 after s.c. tumor implantation as produced by treatment with phenidone and a cyclooxygenase inhibitor along with cytotoxic cancer therapies

| Treatment | Tumor growth delay, days | Number of lung metastases (% large) |
|---|---|---|
| Phenidone/sulindac | 3.1±0.3 | 12 (40) |
| Phenidone/diflunisal | 2.2±0.3 | 12 (48) |
| Phenidone/indomethacin | 3.0±0.4 | 12 (35) |
| Phenidone/mefenamic acid | 0.9±0.3 | 10 (45) |
| CDDP (10 mg/kg) | 4.5±0.3 | 13 (58) |
| Phen/Sulin/CDDP | 10.9±1.1 (2.4)* | 9 (48) |
| Phen/Diflun/CDDP | 8.5±0.4 (1.9)* | 11 (36) |
| Phen/Indo/CDDP | 11.2±0.8 (2.5)** | 8 (45) |
| Phen/Mefen A/CDDP | 9.3±0.7 (2.1)* | 5 (50) |
| Cyclophosphamide (3×150 mg/kg) | 21.5±1.7 | 12 (40) |
| Phen/Sulin/CTX | 45.4±2.9 (2.1)* | 2.5 (32) |
| Phen/Diflun/CTX | 43.2±2.8 (2.0)* | 2.5 (52) |
| Phen/Indo/CTX | 40.9±3.0 (1.9)* | 3.3 (61) |
| Phen/Mefen A/CTX | 40.7±2.8 (1.9)* | 2.5 (46) |
| Melphalan (10 mg/kg) | 2.7±0.3 | 13 (48) |
| Phen/Sulin/PAM | 12.6±0.9 (4.7)** | 9 (48) |
| Phen/Diflun/PAM | 7.9±0.4 (2.9)** | 11 (36) |
| Phen/Indo/PAM | 11.2±0.7 (4.1)** | 10 (40) |
| Phen/Mefen A/PAM | 3.9±0.4 (1.4) | 4 (25) |
| BCNU (3×15 mg/kg) | 3.6±0.4 | 15.5 (53) |
| Phen/Sulin/BCNU | 6.3±0.5 (1.8)* | 11 (36) |
| Phen/Diflun/BCNU | 5.3±0.3 (1.5)* | 15 (39) |
| Phen/Indo/BCNU | 6.7±0.5 (1.9)* | 15 (40) |
| Phen/Mefen A/BCNU | 6.3±0.5 (1.8)* | 11 (40) |
| X-rays (5×3 Gy) | 4.4±0.3 | 15 (40) |
| Phen/Sulin/5×3 Gy | 14.5±1.3 (3.3)** | 11 (36) |
| Phen/Diflun/5×3 Gy | 7.3±0.7 (1.7)* | 14 (36) |
| Phen/Indo/5×3 Gy | 6.9±0.8 (1.6)* | 12 (40) |
| Phen/Mefen A/5×3 Gy | 7.4±0.9 (1.7)* | 11 (36) |

Parentheses indicate an x-fold increase in tumor growth delay as compared with that produced by the antitumor alkylating agent alone
* $P < 0.01$; ** $P < 0.005$

growth delay produced by cyclophosphamide by a factor of 2.0. The modulator combination of minocycline and mefenamic acid increased the tumor growth delay produced by melphalan by a factor of 4.7, whereas the modulator combination of minocycline and sulindac increased the tumor growth delay produced by melphalan by a factor of 4.5. The combination of minocycline and diflunisal produced the greatest modulation of BCNU, increasing the tumor growth delay produced by that drug by a factor of 3.1. The combination of minocycline and sulindac resulted in a 2.8-fold increase in the tumor growth delay produced by BCNU. Daily administration of minocycline and sulindac increased the tumor growth delay produced by fractionated radiation by a factor of 2.8, whereas daily administration of minocycline and diflunisal increased the tumor growth delay produced by fractionated radiation by a factor of 2.1.

The effect of the lipoxygenase inhibitor phenidone along with each of the four cyclooxygenase inhibitors on Lewis lung tumor growth and metastasis is shown on Table 3. The combinations of phenidone with sulindac or indomethacin resulted in about 3 days of tumor growth delay. The most effective modulators of CDDP were phenidone with indomethacin and phenidone with sulindac, resulting in 2.5- and 2.4-fold increases in tumor growth delay, respectively, as compared with that produced by CDDP alone. The most effective modulation of cyclophosphamide was produced by the combinations of phenidone with sulindac and phenodone with diflunisal, which elicited 2.1- and 2-fold increases in tumor growth delay, respectively, as compared with that produced by the drug alone. The combination of phenidone with sulindac and the combination of phenidone with indomethacin resulted in the most effective modulation of melphalan producing 4.7- and 4.1-fold increases in tumor growth delay, respectively, as compared with that induced by the drug alone. Phenidone with indomethacin was most effective in increasing the tumor growth delay produced by BCNU, resulting in a 1.9-fold increase in tumor growth delay as compared with that elicited by the drug alone. Phenidone with sulindac and phenidone with mefenamic acid were also effective modulators of BCNU, resulting in 1.8-fold increases in tumor growth delay as compared with that produced by BCNU alone. The combination of phenidone with sulindac produced the most effective modulation of fractionated radiation resulting in a 3.3-fold increase in the tumor growth delay as compared with that produced by radiation alone.

Those modulators and modulator combinations that produced the greatest increase in the effectiveness of the cytotoxic therapies toward the primary tumor were not always the most effective against metastatic disease (Tables 1–3). None of the single modulators impacted the number of lung metastases found on day 20. Combinations of a cyclooxygenase inhibitor with minocycline produced modest decreases in the number of lung metastases, but the greatest effect of the modulators in the absence of cytotoxic therapy was obtained with combinations of the lipoxygenase inhibitor phenidone with a cyclooxygenase inhibitor. Treatment with the combination of phenidone and mefenamic acid daily for 2 weeks resulted in a decrease in lung metastases from 20 to 10 on day 20 after tumor implantation. Minocycline was the most effective single modulator along with CDDP, cyclophosphamide, and fractionated radiation. In combination therapies including minocycline or phenidone and a cytotoxic treatment, the cyclooxygenase inhibitor mefenamic acid was most effective overall in reducing the number of lung metastases. The most effective therapeutic combinations against metastatic Lewis lung carcinoma were cyclophosphamide with phenidone and sulindac, diflunisal, or mefenamic acid.

Radioimmunoassay was used to determine the serum levels of prostaglandin $E_2$ and leukotriene $B_4$ in mice bearing the Lewis lung tumor with and without treatment with the single modulators and modulator combinations (Fig. 1). The presence of Lewis lung tumor growing in the animals resulted in an increase in serum levels of both prostaglandin $E_2$ and leukotriene $B_4$ as compared with non-tumor-bearing animals. As the tumor became large, the levels of both prostaglandin $E_2$ and leukotriene $B_4$ decreased. The treatment regimen with the single modulators resulted in variable changes in prostaglandin $E_2$ levels, but

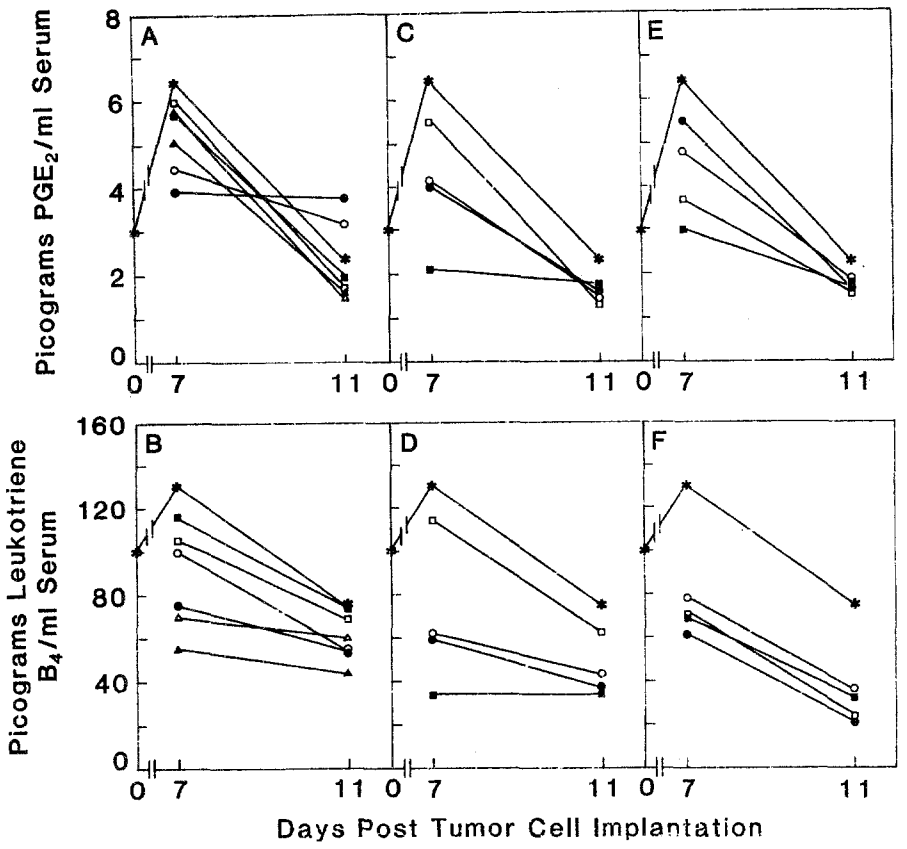


**Fig. 1 A–F.** Serum levels of prostaglandin $E_2$ and leukotriene $B_4$ in animals implanted s.c. with the Lewis lung carcinoma in the hind leg on day 0. **A, B** No treatment ( ), phenidone (●), minocycline (○), mefenamic acid (■), diflunisal (❑), sulindac (▲), indomethacin (△). **C, D** No treatment ( ), minocycline/sulindac (●), minocycline/diflunisal (○), minocycline/indomethacin (■), minocycline/mefenamic acid (❑). **E, F** No treatment ( ), phenidone/sulindac (●), phenidone/diflunisal (○), phenidone/indomethacin (■), phenidone/mefenamic acid (❑). Prostaglandin $E_2$ and leukotriene $B_4$ levels were determined by radioimmunoassay. Each point is the mean of four independent determinations

treatment with phenidone, indomethacin or sulindac decreased circulating levels of leukotriene $B_4$. The combination of minocycline with indomethacin was most effective in reducing serum levels of prostaglandin $E_2$ and leukotriene $B_4$ throughout the observation period. The combinations of minocycline with sulindac or diflunisal were also quite effective. Each of the four cyclooxygenase inhibitors along with phenidone decreased by about one-half the serum levels of leukotriene $B_4$, whereas indomethacin or mefenamic acid in combination with phenidone was most effective in reducing serum levels of prostaglandin $E_2$.

## Discussion

Tumor growth, vascularization, invasion, and metastasis requires activation and/or proliferation of specific host cells and, perhaps, deactivation and/or suppression of others. Metabolic products of the three arachidonic acid pathways are known to be involved in several intercellular signaling processes and, as the networks of intercellular communication are elucidated, continue to be implicated in many of the checks and balances in various tissues [39, 46, 50, 55].

It appears that several major types of non-small-cell lung cancer can generally be regarded as overproducers of prostaglandins (levels of other arachidonic acid products have not been examined in these tumors) [32–35, 40]. It has also been recognized for some time that the murine Lewis lung carcinoma is an overproducer of prostaglandin and that inhibitors of prostaglandin synthesis can influence the growth of that tumor [65–73]. Recently, it has been reported that metabolites of arachidonic acid formed via both the cyclooxygenase and the lipoxygenase pathway are required for the production of collagenase IV and that inhibition of these pathways make human HT-1080 tumor cells noninvasive and nonmetastatic [48]. These studies indicated an important role for the metabolites of arachidonic acid in the production of collagenase IV. It has been recognized for some time that the tetracyclines can inhibit tissue collagenase activity, and tetracycline administration has been used in the treatment of periodontal disease [11] and of gingival collagenolytic activity in diabetes [11, 24] and to inhibit joint deterioration in patients with rheumatoid arthritis [25, 74]. Among the tetracyclines, the semisynthetic derivative minocycline is a relatively potent collagenase inhibitor [12]. Minocycline also has a relatively long circulating half-life of about 12 h and is highly lipid-soluble and may thus have a favorable tissue-penetrating ability [12, 58, 74].

We examined the effect of administration of well-tolerated doses of nonsteroidal antiinflammatory drugs (NSAID) from three structural classes, including the prodrug sulindac, the pyrazoline lipoxygense inhibitor phenidone, and the tetracycline collagenase inhibitor minocycline, on the growth, metastasis, and response to therapy of the Lewis lung carcinoma. Two of the NSAID, diflunisal and sulindac have relatively long circulating half-lives (12–15 h), whereas two others, mefenamic acid and indomethacin, have relatively short circulating half-lives (1.5–5 h) [7]. As a general trend, the NSAID with long circulating half lives were better modulators of cytotoxic cancer therapies for the treatment of the primary disease.

Over the past 10 years the NSAID indomethacin has been studied in several preclinical tumor systems [11, 21–23, 41, 42, 54, 68, 71]. In addition to inhibiting cyclooxygenase, indomethacin has also been shown to inhibit glutathione-S-transferase [45, 64]. Several studies in the Lewis lung carcinoma have examined the relationship between prostaglandin and/or thromboxane production and metastatic potential and have demonstrated that indomethacin is an effective inhibitor of metastasis-related properties in the Lewis lung tumor [21, 68, 71]. Indomethacin has been shown to increase the response of several murine tumors that produce prostaglandins to radiation therapy [22, 23, 41, 42, 47]. The effect of indomethacin was additive with that of misonidazole in murine tumors; however, indomethacin was not an effective modulator of xenografts in nude mice [42].

Sulindac is unique among the NSAID in that it is a prodrug that is reversibly converted via an enzymatic reduction from the parent sulfoxide to the active sulfide. Only the sulfide metabolite is an inhibitor of cyclooxygenase [14, 15]. This metabolic activation step has made sulindac particularly interesting as a potential chemoprevention agent against colon tumors since the ability of colonic bacteria to reduce the parent drug to the active sulfide would result in high concentrations of the active species in the target tissue [14, 53]. Sulindac has been shown to inhibit the rate of development and rate of growth of carcinogen-induced colon and lung tumors in rats and mice [10, 43, 44, 53]. Sulindac treatment has also been reported to cause regression of colon polyps in patients with polyposis and Gardner's syndrome [19, 36, 49, 62].

The modulator combinations produced a greater enhancement of the response of both the primary and the metastatic disease to the cytotoxic therapies than did the single modulators. As a general trend, phenidone/NSAID combinations [6, 13, 26, 29] were more effective than minocycline/NSAID combinations. It is possible that these modulator combinations induce a multiple blockade of biochemical pathways leading to the release of collagenase, thereby inhibiting tumor growth and invasion. In a previous study we found that none of the 11 NSAIDs studied was very cytotoxic and that they generally did not increase the cytotoxicity of antitumor alkylating agents toward EMT-6 cells in culture [59]. EMT-6 tumor-cell survival studies and bone-marrow granulocyte-macrophage colony-forming unit (CFU-GM) survival studies were carried out with seven of the modulators and various doses of cyclophosphamide. Tolmetin, ibuprofen, sulindac, piroxicam, and diflunisal in combination with cyclophosphamide produced increased tumor cell killing as compared with cyclophosphamide alone without producing marked changes in toxicity to the bone-marrow CFU-GM. In EMT-6 tumor growth-delay experiments, none of the six modulators tested affected the growth of the tumors; however, tolmetin, ibuprofen, diflunisal, and sulindac increased the tumor growth delay obtained with standard dose schedules of cyclophosphamide or CDDP. Minocycline in combination with diflunisal or sulindac and either cyclophosphamide, CDDP, or melphalan further increased EMT-6 tumor growth delay. The number of lung metastases and the percentage of lung metastases with diameters measuring greater than 3 mm were reduced by treatment with the minocycline/NSAID combinations alone and were further reduced with the addition of the antitumor alkylating agents [59].

There was no clear correlation between reduction of serum prostaglandin $E_2$ and leukotriene $B_4$ levels and modulator efficacy. Although the mechanism(s) by which these agents operate in the therapeutic setting remains to be defined, further investigation of this modulator strategy, especially in non-small cell lung cancer, is warranted.

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