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Growth Inhibition of Transplantable Murine Colon Adenocarcinoma 38 by Indomethacin*

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Summary. The anti-inflammatory drug indomethacin was tested for antitumor activity against transplantable mouse colon adenocarcinoma 38 (colon 38). Groups of BDF_1 mice (C57BL/6 x DBA/2) were given intraperitoneal injections of this drug beginning on the 6th day after subcutaneous implantation of the tumor and continued for 4 to 8 days. In other groups of mice, identical treatment was delayed until the 16th day after implantation of the tumor. The higher antitumor activity against colon 38 was obtained with earlier initiation of treatment, indicated by decreased growth of the tumor and increased life span of the host. The later initiation of the treatment produced less antitumor activity. The antitumor activity was, however, less than that of 5-fluorouracil, which was used as a positive control drug. The two drugs in combination produced few advantages over 5-fluorouracil alone using the dose schedule designed in the present experiment. Indomethacin treatment significantly reduced prostaglandin E and F levels in the tumor tissue, but 5-fluorouracil did not. It seems likely that the inhibition of prostaglandin biosynthesis by indomethacin underlies the antitumor effect of this drug on colon 38.

Key words: Mouse colon adenocarcinoma 38 – Indomethacin – Prostaglandin

Introduction

The nonsteroid, anti-inflammatory drug indomethacin has been shown to reduce the growth of transplantable tumors (Hial et al. 1976; Lynch et al. 1978; Tashjian et al. 1974; Plescia et al. 1975) and chemically or virally induced primary tumors in animals (Kudo et al. 1980; Narisawa et al. 1981, 1982; Strausser and Humes 1975). The mechanism of inhibition of tumor growth by this drug has not been elucidated clearly. It seems likely, however, that the antitumor activity is closely associated with the inhibition of PG synthesis in tumor tissue by this drug. Prostaglandin E has been suggested to control cell proliferation (Thomas et al. 1974; Lupulescu 1978) and to suppress the cytotoxic activity of lymphocytes and macrophages (Bankhurst 1982; Droller et al. 1978; Schultz et al. 1978; Taffect and Russell 1981). We reported that indomethacin inhibited the growth of tumors ranging from nascent lesions of carcinoma of microscopic dimensions to grossly visible tumors in rats pretreated with a large-bowel carcinogen (Kudo et al. 1980; Narisawa et al. 1981, 1982). These studies prompted us to investigate the antitumor activity of this drug against subcutaneously implanted colon 38, which was successfully established from a 1,2-dimethylhydrazine-induced colon tumor in mice. A number of chemotherapeutic agents have been tested for their activity against this tumor by Corbett and his coworkers (1977). These investigators reported that 5-FU was highly effective against this tumor. Thus, we selected 5-FU as a positive control drug with which indomethacin was compared for antitumor potentiation. Also, PG levels in the tumor tissue of mice treated with indomethacin were measured to assess the pharmacologic activity of this drug on prostaglandin synthesis.

Materials and Methods

Animals and Tumor

Adult male BDF_1 (C57BL/6 x DBA/2) mice were used for the experiments and C57BL/6 mice were used for maintaining the tumor. Both strains of mice were obtained from Charles River Co., Atsugi, Japan. The mice had free access to CL-2 laboratory chow and tap water throughout the experiment and they were weighed once a week. Colon 38 was supplied by the Cancer Chemotherapy Center (Japanese Foundation for Cancer Research, Tokyo, Japan) and was

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Abbreviations: Colon 38 = transplantable mouse colon adenocarcinoma 38; 5-FU = 5-fluorouracil; PG = prostaglandin

Table 1. Antitumor effects of indomethacin and 5-FU on subcutaneously implanted colon 38

Treatment groups	Treated from day	MDD[b] (days)	%ILS[b]	T–C value[b] (days)
$Indo_1$ (n=7)	6– 9	53	71	3
$Indo_2$ (n=10)	6–13	65	110	8
$Indo_3$ (n=10)	16–23	44	42	4
$5\text{-}FU_1$ (n=7)	6– 9	94	203	13
$5\text{-}FU_2$ (n=10)	6–13	84	171	18
$5\text{-}FU_3$ (n=10)	16–23	63	103	14
Indo/5-FU (n=10)	6–13	82	165	17
Untreated (n=10)		31		

[a] Mice received a daily intraperitoneal dose of 2.5 mg/kg body weight of indomethacin in groups $Indo_{1-3}$ and of 20 mg/kg body weight of 5-FU in groups $5\text{-}FU_{1-3}$ during the periods listed. Mice in group Indo/5-FU received separate intraperitoneal administrations of the same doses of indomethacin and 5-FU

[b] MDD = median day of death; % ILS = % increase of life span; T–C value = tumor-growth delay. Values were calculated using formulas described in text

passaged in the mice as described below. For the experiment, the tumor was implanted subcutaneously by trocar into the axillary region of the mice. The trocar pieces weighed approximately 20 mg (10^7 tumore cells, Corbett et al. 1977).

Antitumor Treatment and Method of Evaluation

Indomethacin (Nippon Merck-Banyu Co., Tokyo, Japan) was used as a 0.075 % solution in a 0.3% aqueous solution of methylcellulose and 5-FU (Kyowa Hakko Kogyo Co., Tokyo, Japan) as a 0.6% solution in 0.9% NaCl solution. The submaximal dosage of indomethacin for chronic toxicity in mice and the optimal dosage of 5-FU for antitumor activity against colon 38 (Corbett et al. 1977) were selected. Each treated mouse received a daily intraperitoneal administration of 2.5 mg/kg body weight of indomethacin and/or 20 mg/kg body weight of 5-FU. The treatment for 4 or 8 days in each of five experimental groups was started on the 6th day after tumor implantation, when the number of tumor cells was relatively small and gross tumors had not appeared. For another two experimental groups of mice the same treatment for 8 days was delayed to begin on the 16th day, when the implanted tumors were growing and visible. Ten or seven mice were used in each treatment group. The detailed experimental design is shown in Table 1. Ten untreated mice served as a control group.

The tumors were measured with a caliper once a week and the mice were observed daily until the time of death. Tumor weight was calculated from two-dimensional measurement as described (Corbett et al. 1977): Tumor weight (mg) = a(mm) × b^2(mm)/2, where a is the length and b is the width of the tumor. Antitumor activity was evaluated by (a) percent increase in host life span (% ILS) = 100 × [the median day of death (MDD) of the treated mice minus the MDD of the control mice]/MDD of the control mice and (b) tumor-growth delay (T-C, days) = the median time required for the treatment-group tumors to reach a predetermined size (750 or 1,000 mg) minus the median time required for the control-group tumors to grow to the same size. Tumor-free survivors were excluded from these calculations. All transplanted tumors and the metastatic involvement in the lungs, liver, lymph nodes, and other organs were inspected macroscopically at autopsy. All tumors and organs considered abnormal were examined histologically after standard processing.

Measurement of Prostaglandins

Indomethacin or 5-FU at the dose levels described above was administered intraperitoneally twice a day from the 14th to the 16th day to each of four mice having an implanted and growing gross tumor. Six hours after the last injection of these drugs, the mice were killed by cervical dislocation and subcutaneous tumors were excised and weighed. One-half of each excised tumor was immediately frozen and stored at −70 °C until extraction for measurement of PG. The other half of the tumor was placed in 4% formalin for histologic examination. The histologic sections were stained with hematoxylin and eosin. The levels of prostaglindin E (E_1 and E_2) and prostaglandin F ($F_{1\alpha}$ and $F_{2\alpha}$) in the extracts of homogenized tumor tissue were measured by radioimmunoassay by methods described by Jaffe et al. (1973) at Kitazato Biochemistry Laboratory, Sagamihara, Kanagawa, Japan. The data were analyzed statistically using the Student's t test and χ^2 test.

Results

All the mice tolerated the administration of indomethacin and/or 5-FU well and the body-weight gains of the mice receiving treatment were not different from those of untreated control mice during the course of the experiment before the mice had large growing tumors and were in a condition of cachexia. Indomethacin showed a high antitumor effect on the growth of colon 38. The mean tumor weights of colon 38 in mice treated with indomethacin or 5-FU for 4 or 8 days begun on the 6th day after tumor inoculation are shown in Fig. 1. It shows that the tumor growth was suppressed significantly by 8-day indomethacin treatment in group $Indo_2$ and by 4- or 8-day 5-FU treatment in groups $5\text{-}FU_1$ and $5\text{-}FU_2$, respectively, throughout the experiment compared with untreated control mice ($p < 0.025$). This inhibitory effect was less marked in mice receiving 4-day indomethacin treatment in group $Indo_1$. The 5-FU treatment for either 4 or 8 days was more effective in the reducing tumor growth than indomethacin treatment. The tumor-growth delay (T-C value) was 3 or 8 days in mice of groups $Indo_1$ or $Indo_2$ receiving indomethacin for 4 or 8 days, respectively (Table 1). Thus, indomethacin significantly suppressed tumor growth in the early stage of the disease only when the treatment was continued for 8 days. The 5-FU had a cytocidal effect during the early stage of tumor growth and, in these groups ($5\text{-}FU_2$ and $5\text{-}FU_1$), the treatment for both 8 and 4 days resulted in longer delays of tumor growth of 18 and 13 days, respectively. Furthermore, in group $5\text{-}FU_2$, no mice had palpable tumors 2 weeks after implantation (Fig. 1). The median survival time of groups of mice receiving either indomethacin and/or 5-FU was significantly greater than that of untreated control mice ($p < 0.05$; Table 1 and Fig. 2). The %ILS was 71% and 110% in groups treated with indomethacin for 4 and 8 days, respectively, and 203% and 171% in groups treated with 5-FU for

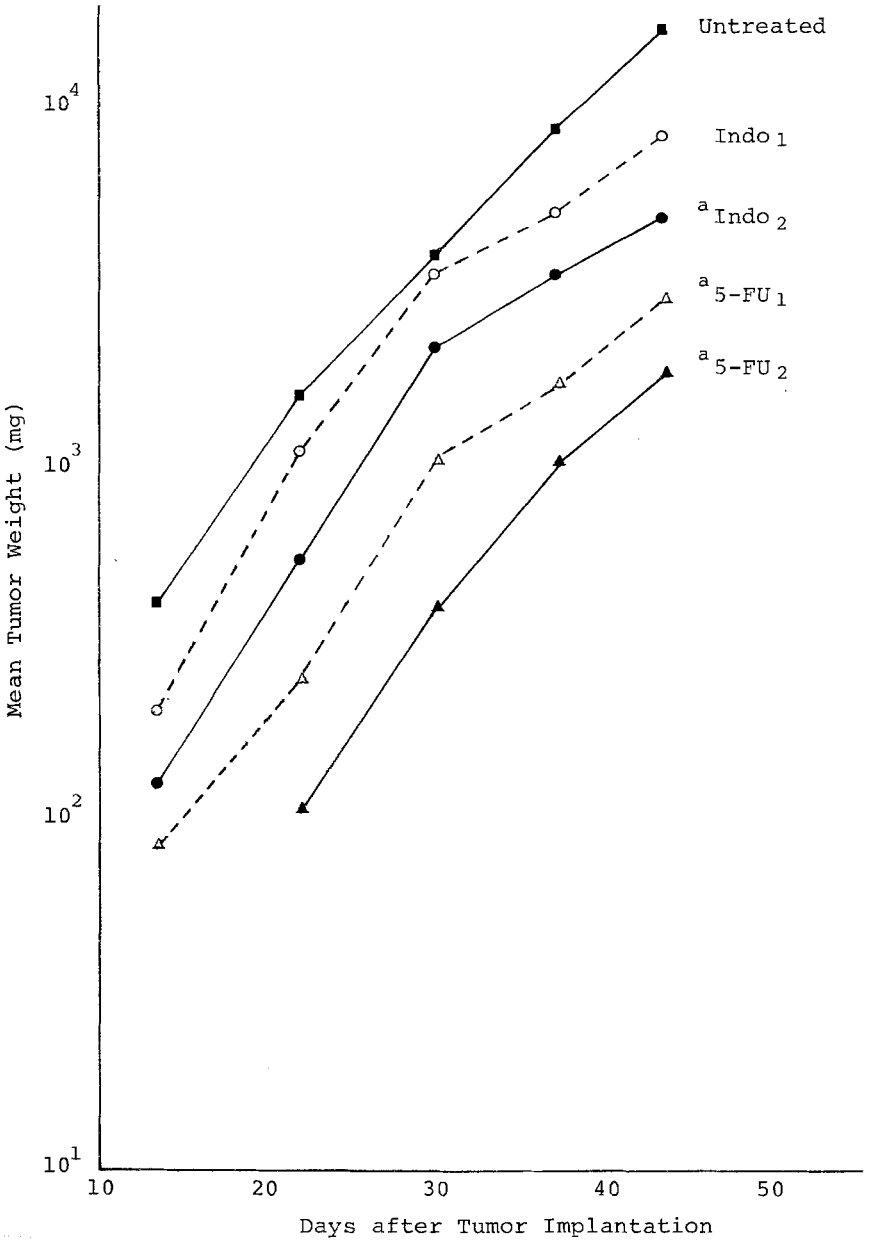

Fig. 1. Effect of indomethacin and 5-FU on tumor growth in mice with transplanted colon 38. Treatment was begun on the 6th day after tumor implantation. Mice were treated with an intraperitoneal injection of 2.5 mg/kg body weight of indomethacin for 4 days (group $Indo_1$) or 8 days (group $Indo_2$) and of 20 mg/kg body weight of 5-FU for 4 days (group 5-FU_1) or 8 days (group 5-FU_2). Control mice received no treatment (Untreated). The mice in group 5-FU_2 showed no palpable tumors 2 weeks after implantation. a = The treatment in groups $Indo_2$, 5-FU_1 and 5-FU_2 significantly reduced the tumor growth at all times compared with the group Untreated; $p<0.025$

4 and 8 days. The 5-FU effectively diminished the tumor growth and also produced a significant effect on the survival time even in mice in which the treatment was begun during the later stage of the disease in group 5-FU_3 (Table 1). In group 5-FU_3, the mean tumor weight was reduced by 62% after treatment by 5-FU for 8 days begun on the 16th day after implantation and the decrease was significant, compared to the control group (data not included). However, the mean tumor weight increased rapidly in the next week. The treatment with indomethacin delayed until the 16th day after implantation in group $Indo_3$ had less effect on tumor growth and survival time (Table 1). Thus, the treatment with indomethacin and 5-FU in combination was not statistically more effective than the treatment with 5-FU alone (Table 1).

There were no tumor-free survivors in any groups of mice. At the time of death, all mice showed large subcutaneous tumors. Multiple lung metastases were observed in three mice of group $Indo_1$, five of group 5-FU_2, one of group Indo/5-FU, and three of group 5-FU_1. All of these metastatic tumor-bearing mice except one survived longer than 80 days after implantation. One metastatic tumor was found in the liver of one mouse of group 5-FU_1. There were no lymph nodes involved.

Indomethacin treatment significantly reduced the amount of prostaglandins E and F in the tumor tissues,

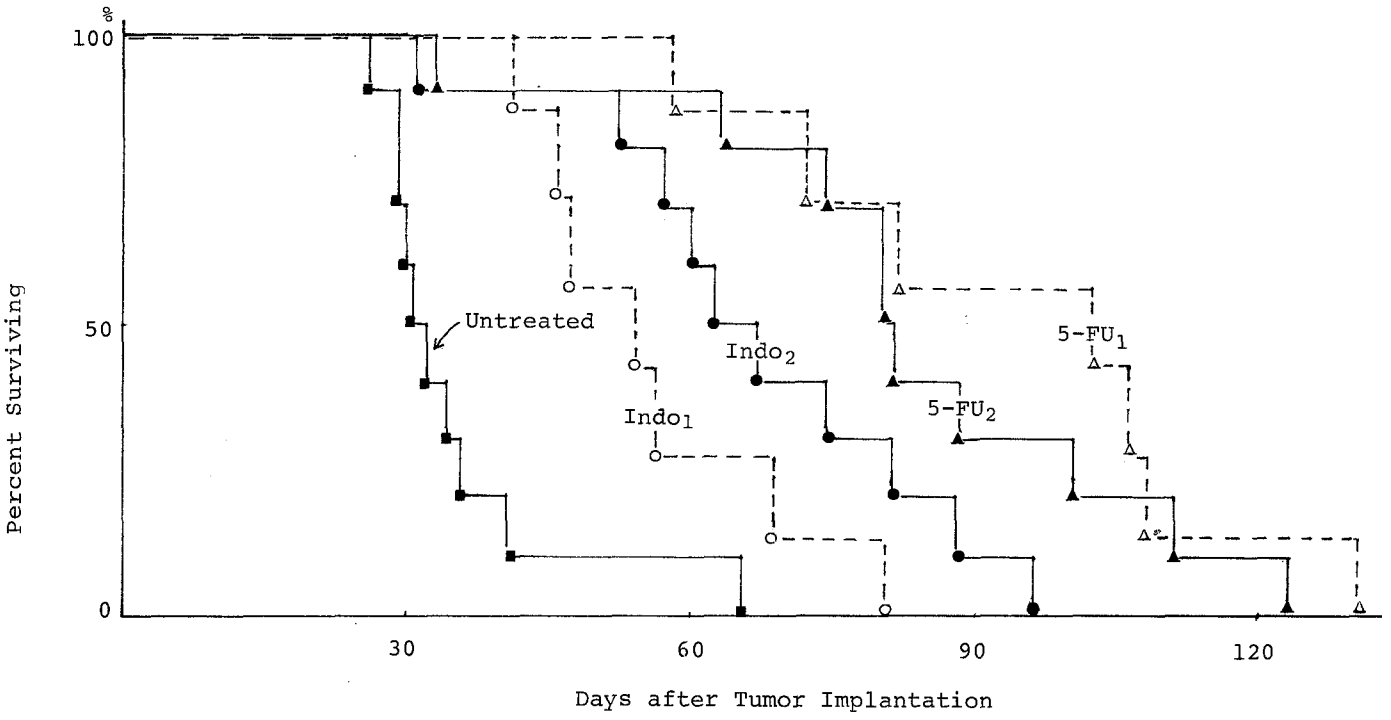

Fig. 2. Effect of indomethacin and 5-FU on survival of mice with transplanted colon 38. Treatment was begun on the 6th day after tumor implantation. Mice were treated with an intraperitoneal injection of 2.5 mg/kg body weight of indomethacin for 4 days (group $Indo_1$) or 8 days (group $Indo_2$) and of 20 mg/kg body weight of 5-FU for 4 days (group $5\text{-}FU_1$) or 8 days (group $5\text{-}FU_2$). Control mice received no treatment (Untreated)

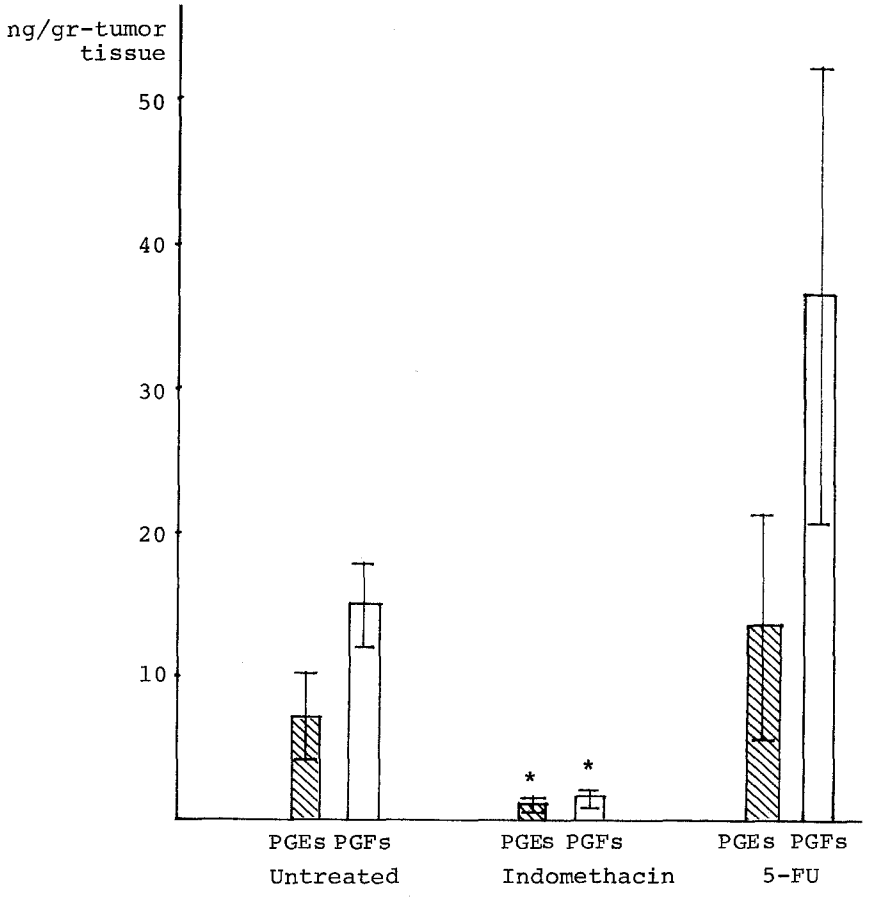

Fig. 3. Effect of indomethacin and 5-FU on the levels of PGE (E_1 and E_2), and PGF ($F_{1\alpha}$ and $F_{2\alpha}$) in colon 38 tissue. BDF_1 mice were subcutaneously implanted with 20 mg colon 38. The tumors of four mice in each group were removed on the 16th day after 2-day treatment with indomethacin or 5-FU. The prostaglandins in the tumor extracts were measured by radioimmunoassay. Bars represent S.D. * Significantly different from Untreated and 5-FU groups; $p<0.05$ or better

compared with untreated control mice (Fig. 3). Prostaglandin levels in the tumors of mice treated with 5-FU, however, were higher than in untreated controls, but the difference was statistically insignificant. There was no relationship between the amount of PG and tumor weight in each mouse. On microscopic examination of these tumors excised for PG measurement, no degenerative or necrotic area was observed and lymphocytes and macrophages were scantily involved in the tumors of mice treated with indomethacin and 5-FU, as well as untreated mice. Thus, we found that no discernible changes or therapeutic effects on gross and microscopic findings due to 2-day administration of indomethacin or 5-FU were observed in the histologic sections of the tumors excised just after the completion of short-term treatment.

Discussion

In recent years, there have been a number of reports concerning the ability of nonsteroid anti-inflamatory agents, such as indomethacin, to inhibit the growth of a variety of transplantable solid and ascites tumors in mice (Bennett et al. 1979; Fulton and Levy 1980; Gatenby et al. 1980; Hial et al. 1976; Tashjian et al. 1974) and rats (Trevisani et al. 1980). Furthermore, indomethacin suppressed the growth of Moloney sarcoma virus-induced primary tumors in mice (Strausser and Humes 1975) and the development of chemical carcinogen-induced primary colonic carcinomas in rats (Kudo et al. 1980; Narisowa et al. 1981, 1982). The present study showed clearly that indomethacin in pharmacologic doses inhibited the tumor growth and lengthened the survival time of colon 38-bearing mice. The antitumor activity was dependent on the stage of the disease and on the duration of indomethacin administration. In the present series of experiments, the greater antitumor activity, indicated by T-C value and %ILS, was associated with longer duration of indomethacin treatment and resulted in smaller tumors. However, the antitumor activity of this drug against colon 38 was less than that of 5-FU, which was selected as a positive control drug based on reports that it is the most effective chemotherapeutic agent against this tumor (Corbett et al. 1977).

The mechanism of inhibition of tumor growth by these drugs has been investigated from several aspects. It is well known that they are able to reduce endogenous PG biosynthesis (Lynch et al. 1978; Strausser and Humes 1975; Trevisani et al. 1980). Relatively high concentrations of PG were reported to be contained in experimental tumors (Hial et al. 1976; Tashjian et al. 1974; Trevisani et al. 1980) and in human carcinomas, including large-bowel carcinoma (Bennett et al. 1977). Prostaglandin could inhibit the natural and antibody-dependent lymphocyte cytotoxicity (Bankhurst 1982; Droller et al. 1978) and also the nonspecific macrophage-mediated tumoricidal activity (Schultz et al. 1978; Taffect and Russell 1981). Thus, indomethacin may restore or augument lymphocyte- and macrophage-mediated immunopotentiation (Boorman et al. 1982; Maca and Panje 1982). This evidence suggests that the antitumor activity of indomethacin on colon 38 used in the present experiment derives, at least in part, from the inhibition of endogenous PG synthesis. The decrease in synthesis of PG in colon 38 by indomethacin was corroborated by this study. However, the exact mechanism by which indomethacin suppresses the growth of colon 38 remains to be investigated further. Indomethacin, aspirin, Corynebacterium parvum, or a combination of these agents can significantly reduce the number and weight of lung metastases from the subcutaneously implanted Dunn osteosarcoma, Lewis lung carcinoma, and B16 melanoma in mice (Gatenby et al. 1980). In our study, the incidence and number of lung and liver metastases were lower, though insignificantly, in groups treated with indomethacin or the combination of indomethacin and 5-FU than in groups treated with 5-FU alone.

It has been reported that indomethacin arrests the growth of rat hepatoma and human fibroblast cultures in the G_1 phase of the cell cycle (Bayer et al. 1979; Hial et al. 1977). Bennett et al. (1979, 1982) reported that the addition of the nonsteroidal, anti-inflammatory drug flurbiprofen or indomethacin to radiotherapy or chemotherapy could produce more beneficial effects than either therapy alone. However, the results from the present investigation do not indicate any beneficial effects of combination therapy with indomethacin and 5-FU compared with treatment with 5-FU alone. Hofer et al. (1980) also reported that the combination of indomethacin with a chemotherapeutic agent did not cause any significant change in the mean viable cell count of B16 melanoma in mice. Macrophages activated by immunopotentiator release PGE (Humes et al. 1977; Schultz et al. 1978; Taffect and Russell 1981), so PGE could act locally in negative feedback inhibition of the activated state. Taffect et al. (1981) also reported that treatment of bacterial lipopolysacchride-stimulated macrophages with indomethacin prevented PGE synthesis and promoted its cytolytic activity. Lynch and Salomon (1979) noted that oral administration of indomethacin enhaced BCG treatment and augmented the activity of Corynebacterium parvum against transplantable fibrosarcoma in mice. Thus, indomethacin combined with chemotherapeutic and immunotherapeutic agents might produce great antitumor effect and antimetastatic activity against several tumors. Further studies are needed to investigate various regimens of indomethacin treatment, as well as to

elucidate the mechanism of antitumor activity of this drug before clinical trials for human cancers can be initiated.

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