ELSEVIER

Biochemical Pharmacology 61 (2001) 565–571

Biochemical Pharmacology

Comparative effects of indomethacin on cell proliferation and cell cycle progression in tumor cells grown *in vitro* and *in vivo*

Yaniv Eli[a], Fiorenza Przedecki[a], Galit Levin[a], Na'am Kariv[b], Amiram Raz[a,*]

[a]*Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Avis 69978, Israel*
[b]*Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel*

Received 12 January 2000; accepted 9 August 2000

Abstract

Considerable research effort is currently being directed towards understanding the mechanisms mediating the antiproliferative effects of non-steroidal anti-inflammatory drugs (NSAIDs) and, more recently, of cyclooxygenase (COX)-2 inhibitors as well. A key question is whether NSAIDs (excluding sulindac) exert their anticarcinogenic effects *in vivo* by a mechanism that is dependent on their capacity to inhibit COX activity. Some studies with cultured tumor cells *in vitro* have argued against such a linkage, showing that NSAIDs inhibit cell replication and/or augment apoptosis only at concentrations that exceed those required to inhibit COX activities 10- to 100-fold. The significance of these results for the observed anticarcinogenic effects of NSAIDs *in vivo* has not yet been evaluated. We addressed this question by comparing, for the same tumor cells, the effects of the NSAID indomethacin on cell growth parameters when the cells were grown in culture to the effects seen in the *in vivo* growing tumor in the mouse. Indomethacin added to cultured Lewis lung carcinoma cells exerted a potent antiproliferative effect (^{3}H thymidine assay) and reduced cell viability (MTT[3-(4,5-dimethyl(thiazol-2-yl)-2,5 diphenyl tetrazolium bromide] assay) at low doses (10–20 μM) in parallel with its inhibitory effect on cellular cyclooxygenase. These effects of indomethacin appeared to arise from a clear antiproliferative shift in the profile of the cell cycle parameters towards a reduced percentage of cells at the S and G_2/M phases, together with an increased percentage of cells at the G_1 phase. Significantly, similar results were seen when indomethacin was given *in vivo* at the low dose of 2 mg per kg/day, which blocked blood platelet COX activity and at the same time produced a delay in tumor growth initiation and attenuation of apparent primary tumor growth as well as growth of lung metastases. These results thus provide strong support for the notion that COX inhibition is a major determinant in the antitumorigenic effect of indomethacin *in vivo*.

Keywords: Cyclooxygenases; NSAIDs; Indomethacin; Tumor cells; Cell cycle; Cell proliferation

1. Introduction

Previous studies have demonstrated that NSAIDs given *in vivo* to rodents [1–3] and humans [4] can inhibit tumor growth. The biochemical mechanism generally ascribed to this effect is the inhibition of both COX enzymes. In addition to NSAIDs, which inhibit COX-1 and COX-2, selective COX-2 inhibitors were also found to attenuate tumor growth in some animal tumor models [5,6]. An explanation for the antineoplastic properties of NSAIDs was first suggested in 1972 by Adolphe *et al*. [7], who reported that certain NSAIDs were capable of inhibiting the proliferation of cultured HeLa cells by causing cell cycle arrest. Recently, several groups have shown that certain NSAIDs induce apoptosis of tumor cell lines [8–13]. The caveat of most of these results is that they are based on *in vitro* studies with cultured cells which are treated with NSAIDs at concentrations 10- to 100-fold higher than those required for inhibition of both COX enzymes. Nevertheless, these findings led some investigators [11–13] to suggest that non-COX mechanisms are involved at least in part in the antineoplastic effects of NSAIDs seen *in vivo*. However, these suggestions remain to be validated, since the exceedingly high concentrations employed in cell culture experiments far exceed the systemic and tumor concentrations obtained when the drugs are given in an effective anticarcinogenic dose *in vivo*. To directly address this issue, we compared the

* Corresponding author. Tel.: +972 3 640 9442; fax: +972 3 640 6834.

E-mail address: amiraz@post.tau.ac.il. (A. Raz).

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; MTT, 3-(4,5-dimethyl(thiazol-2-yl)-2,5 diphenyl tetrazolium bromide; HBSS, Hanks' balanced salt solution; and LL, Lewis lung.

0006-2952/01/$ – see front matter
PII: S0006-2952(00)00578-5

effect of the NSAID indomethacin when added *in vitro* to cultured tumor cells to its effect *in vivo* in the same cells when they were freshly isolated from tumors grown in mice. The results obtained demonstrate that indomethacin given *in vivo* to mice at a low dose of 2 mg per kg/day drastically inhibits systemic COX activity in parallel with inhibition of tumor growth by a mechanism that involves reduced tumor cell proliferation as well as increased apoptosis.

2. Materials and methods

2.1. Studies with cells in culture

2.1.1. Cell culture

3LL-D122, a highly metastatic variant of mouse LL carcinoma cells [14], was kindly provided by Dr. Lea Eisenbach of the Department of Cell Biology, Weizmann Institute of Science (Rehovot, Israel). The cells were grown in culture in DMEM containing 10% PBS, 2% glutamine, 1% non-essential amino acids, 1% Na-pyruvate, and penicillin/streptomycin. Cells were passed at 80% confluency by short exposure to 0.25% trypsin–EDTA. For experiments with indomethacin, an 0.15 M stock solution was prepared in 1 M Tris–HCl, pH = 8.2, and then diluted with culture medium to a concentration of 1–15 mM, from which 10-μL aliquots were added to 1 mL of culture medium to obtain the final concentrations indicated.

2.1.2. [^{3}H]Thymidine incorporation

Subconfluent cells were cultured in 6-well plates and incubated for 24 hr with 5 uCi of [^{3}H]thymidine. The cells were then washed 3 times with HBSS, lysed with 1 N NaOH, and the lysate counted by liquid scintillation.

2.1.3. MTT cell viability assay

This method provides a quantitative measure of the number of cells with metabolically active mitochondria and is based on the mitochondrial reduction of a tetrazolium bromide salt (MTT) with resulting characteristic absorption at around 450 nm. Cells were plated in a 96-well plate at 10^4/well and after 24- to 48-hr incubation, MTT was added (final conc. 1 mg/mL) for 4 hr and the absorbance at 450 nm read by an ELISA plate reader. This absorbance was linearly proportional to the number of live cells with active mitochondria.

2.1.4. Cell sorter analysis

Cells were cultured as described. Harvested cells were diluted to contain approx. 10^6 cells/mL. DNA distribution analysis was performed after digestion of cells by adding 50 μL of Triton X-100, followed by vortex and staining with propidium iodide (Sigma) (50 μL of 1 mg/mL solution added). Analysis was done in a Becton Dickinson FACSorter using the Cell Quest Software.

2.1.5. Assay of cellular COX activity

Cultured cells were treated with indomethacin (0.1–50 μM) for 30 min, after which arachidonic acid was added (15 μM final concentration) and the cells incubated for 15 min. The media were analyzed by radioimmunoassay using anti-PGE_2 (prostaglandin E_2) antisera from Sigma. Assay of platelet COX-1 activity was performed by withdrawing blood from the mice by orbital eye bleeding and allowing it to clot at 37° for 15 min. The resulting serum was assayed for thromboxane B_2 (TXB_2) by radioimmunoassay using anti-TXB_2 antisera from Sigma.

2.2. Mouse footpad primary tumor model

Male C57BL/6J mice, aged 8–10 weeks, were obtained by in-house breeding from the Tel Aviv University Animal Care Facility. Food and water were supplied *ad lib*. Mice were kept in rooms at constant temperature and humidity and weighed weekly. LL carcinoma cells were grown and passed in culture as described above. For inoculation in the paw, cells were harvested and suspended in HBSS containing Ca^{2+} and Mg^{2+} at a concentration of 6.7×10^6/mL, and 30 μL of the suspension was injected s.c. into the right footpad of the mice using an insulin syringe with a 28 G needle. Progression of tumor growth was determined by measuring tumor dimensions (footpad thickness and width) with a caliper every 3–4 days and calculating tumor volume according to the formula 0.52 × thickness squared × width. In some groups, indomethacin (prepared as a 100X stock of 1 mg/mL in 0.25 M Tris–HCl, pH = 8.5) was added in the drinking water at a final concentration of 10 μg/mL. Fresh drinking water containing indomethacin was prepared daily.

2.3. Ex vivo *studies with cells isolated from* in vivo *growing tumors*

Mice were killed by cervical dislocation, the footpad tumor punctured, and the tumors cells' soft mass squeezed and weighed. Approx. 100 mg of tumor mass was resuspended in 1 mL of HBSS using a 200-μL pipette tip. The suspension was allowed to settle for 5 min, during which tissue debris and connective tissue settled. The upper layer cell suspension was aspirated and diluted to contain approx. 10^6 cells/mL. Cell sorter DNA distribution and MTT cell viability assays were performed as described above. Cellular COX activity was determined by incubating the cells with arachidonic acid (15 μM) for 15 min and the supernatant assayed for prostaglandin E_2 radioimmunoassay.

2.4. The natural metastases model

In some experiments, tumor-bearing mice in which the tumor reached a volume of 0.2–0.25 mL were anesthetized with a mixture of ketamine–HCl and xylazine and the footpad with the tumor aseptically amputated. The mice recovered quickly and did not appear to be significantly hindered

in their movements. As demonstrated previously in this type of model [15], there is acceleration of metastatic growth following the removal of the primary tumor. In our studies, the control mice developed substantial lung metastases 25–30 days after tumor amputation and began to die soon afterwards. The time-span between the date of amputation and the date of death of the first mouse to die in the control group was recorded, and all mice in all groups were killed individually at the same time interval after amputation and the lung plus metastatic tissue isolated and weighed.

2.5. The direct metastases model

In contrast to the previous model, where metastases develop from tumor cells originating from the primary tumor, the second model we tested is a direct metastasis model in which tumor cells are injected into the bloodstream and, with this cell type, lodge preferentially in the lungs. Cultured cells were harvested, diluted in HBSS to give 1.67×10^6 cells/mL, and 0.3 mL (5×10^5 cells) injected into the tail vein. Control mice developed metastases in 25–30 days and began to die shortly thereafter, at which time animals in all groups were killed and the lungs plus tumors isolated and weighed.

2.6. Statistical analysis

Evaluation of statistical significance between values in different groups was done using the Student's *t*-test, except for the data presented in Fig. 5C, where ANOVA was used for repeated measurements.

3. Results

3.1. Effect of indomethacin on LL carcinoma cells grown in culture

3.1.1. Effect of indomethacin on cell proliferation and cell viability

Cultured cells at approx. 50% confluency were incubated with various doses of indomethacin and [^{3}H]thymidine for 48 hr. The cells were then washed twice with HBSS and the radioactivity in the cells counted. Indomethacin at a dose of 10 μM inhibited prostaglandin E_2 synthesis from added arachidonate by >90% (data not shown) and also reduced [^{3}H]thymidine uptake by approx. 50% (Fig. 1A). Analysis of cell viability using the MTT assay showed that indomethacin significantly inhibited cell viability at 20 μM, with 50% inhibition at approx. 60 μM (Fig. 1B).

3.1.2. Effect of indomethacin on cell cycle parameters

Cell sorter analysis of isolated cultured tumor cells grown to approx. 50% confluency revealed a rapid growth rate which was attenuated dose-dependently by indomethacin, as evidenced by the decline in the percentage of cells in the G_2/M phase with the concomitant increase in the percentage of cells in G_1 (Fig. 2). Significantly, a pre-G_1 apoptotic peak was not seen in control cultured cells (Table 1) nor in cells treated with indomethacin up to a concentration of 80 μM (data not shown). Overall, the data indicate that indomethacin added to cultured LL carcinoma tumor cells in concentrations of up to 20 μM exerts significant net

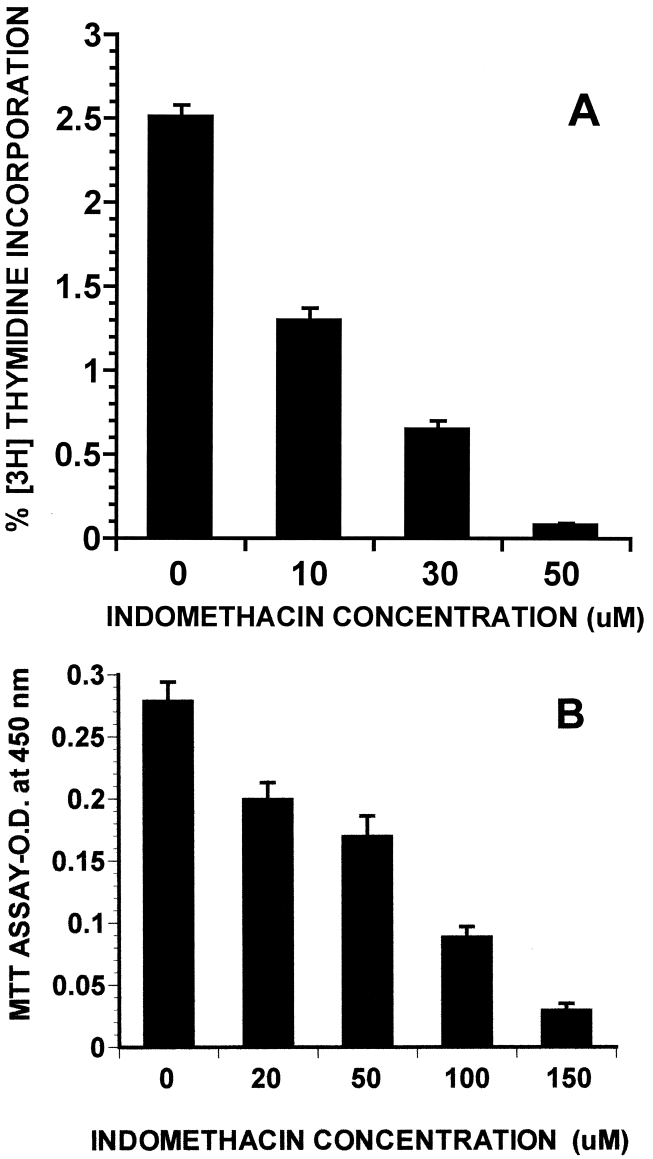

Fig. 1. Dose-dependent effects of indomethacin on [^{3}H]thymidine incorporation and on cell viability (MTT accumulation) in cultured LL carcinoma cells. (A) Cells were plated on 6-well plates and grown to approx. 50% confluency (generally 36–48 hr). The cells were then treated with various doses of indomethacin (10–50 μM) for 24 hr, after which cellular incorporation of [^{3}H]thymidine was determined. Values are means ± SEM of 6 wells and are significantly different from each other (*t*-test, $P < 0.01$). (B) LL carcinoma cells were grown on a 96-well plate for 24 hr to reach approx. 50% confluency. Various doses of indomethacin were then added and the incubation continued for 24 hr, after which the cells were treated with MTT and assayed for cellular MTT uptake. Values are means ± SEM of 15 wells. All values are significantly different (*t*-test, $P < 0.05$) from the value obtained without added indomethacin.

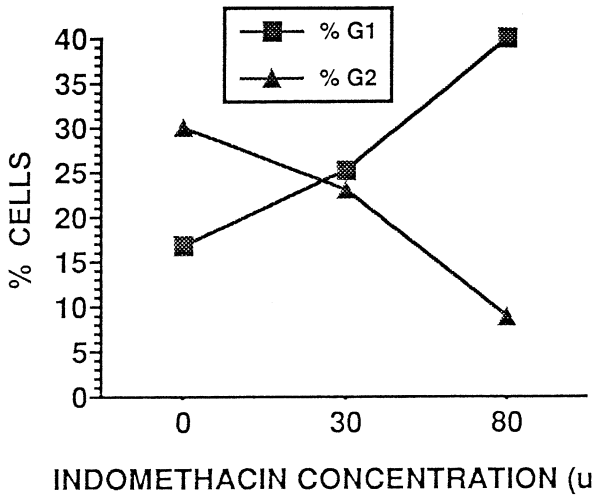

Fig. 2. Dose-dependent modulation of cell cycle progression by indomethacin. LL carcinoma cells were grown in T-75 flasks as described in Fig. 1. Indomethacin at different doses was then added and the cells incubated for 24 hr. The cells were then harvested, washed with HBSS, and processed for cell cycle analysis. Values are means ± SEM from 4 flasks.

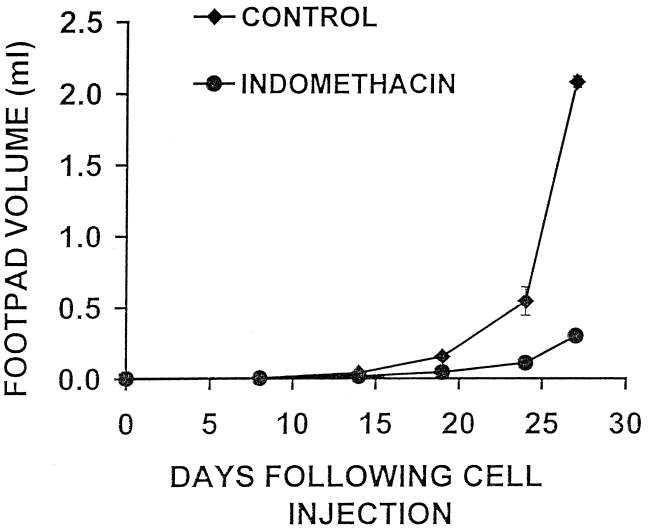

Fig. 3. Low dose indomethacin inhibits tumor growth *in vivo*. Mice were given drinking water containing 10 μg/mL of indomethacin beginning one day before inoculation with LL carcinoma cells and continuing until the termination of the experiment. Tumor growth in the inoculated paw was followed and tumor volume measured every 3–4 days. Net tumor volume was calculated by subtracting the volume of the contralateral uninjected paw. Values are means ± SEM of 10 mice per group.

antipoliferative effect by reducing both the rate of mitosis and cell viability.

3.2. Effect of in vivo administration of indomethacin on overall tumor growth and tumor cell viability/apoptosis in tumor-bearing mice

3.2.1. Effect of indomethacin on tumor growth and cell viability

Indomethacin given at a concentration of 10 μg/mL in the drinking water (calculated to be approx. 2 mg/kg/day) inhibited platelet COX-1 (thromboxane B_2 formation) by >95% (data not shown) and significantly delayed the onset of tumor growth and the initial growth rate of the footpad tumors (Fig. 3). Tumor cells isolated from the footpad tumors were prepared for MTT viability assay as described in Materials and Methods and in the legend to Fig. 1B. Indomethacin treatment produced a marked decline of approx. 85–90% in the viability of *in vivo* growing tumor cells as determined by the MTT assay (Fig. 4A).

Table 1
Comparative effects of indomethacin on the proliferative/apoptotic profile of tumor cells grown *in vitro* and *in vivo*

Cell cycle phase	% cells in cell cycle phase		
	Cultured cells 50% confluent	Cultured cells 80% confluent	*In vivo* growing footpad tumor cells
G_1	19.7 ± 1.4*	31.9 ± 2.2**	46.4 ± 4.3***
S	49.9 ± 3.1*	49.2 ± 4.1*	32.6 ± 2.5**
G_2/M	30.3 ± 2.7*	18.8 ± 2.1**	12.1 ± 1.7***
Apoptosis	0*	0*	8.9 ± 1.2**

Cell sorter analysis was performed on LL carcinoma cells cultured *in vitro* and harvested when either in log phase (approx. 50% confluent) or approaching confluency (80% confluent) in comparison to cells isolated from an *in vivo* growing tumor. Values are means ± SEM of 6 wells or 6 footpad tumors.

For each cell cycle phase, values with a different superscript are significantly different from each other (Student's *t*-test, $P < 0.01$).

3.2.2. Effect of indomethacin on cell cycle parameters

In contrast to the cell cycle profile seen with cultured tumor cells, the *in vivo* growing tumor cells did contain a substantial number of pre-G_1 apoptotic cells in the range of 7–17% of total cells depending on the size of the tumor at the time of its excision (Table 1). Indomethacin treatment significantly elevated the number of apoptotic cells in the tumor (Fig. 4B). Furthermore, tumor cells from indomethacin-treated mice showed a decline in the number of cells in the G_2/M and S phases and a concomitant increase in the percentage of cells in the G_1 phase (Fig. 4B). Thus, the overall effect of *in vivo* low dose indomethacin treatment is antitumorigenic by virtue of its both accelerating apoptosis and causing a G_1 arrest.

3.2.3. Inhibition by indomethacin of the growth of lung metastases and tumor-associated cachexia

We also evaluated the antimetastatic effect of indomethacin using both the natural and the direct metastases models. In the natural metastases model, the primary tumor is amputated when it is relatively small and not life-threatening. Examination of mouse lungs at this stage reveals no visible macro- or micrometastases. If, however, the animals are kept for an additional 25–30 days, they develop massive metastatic tumors in their lungs. The overall model is de-

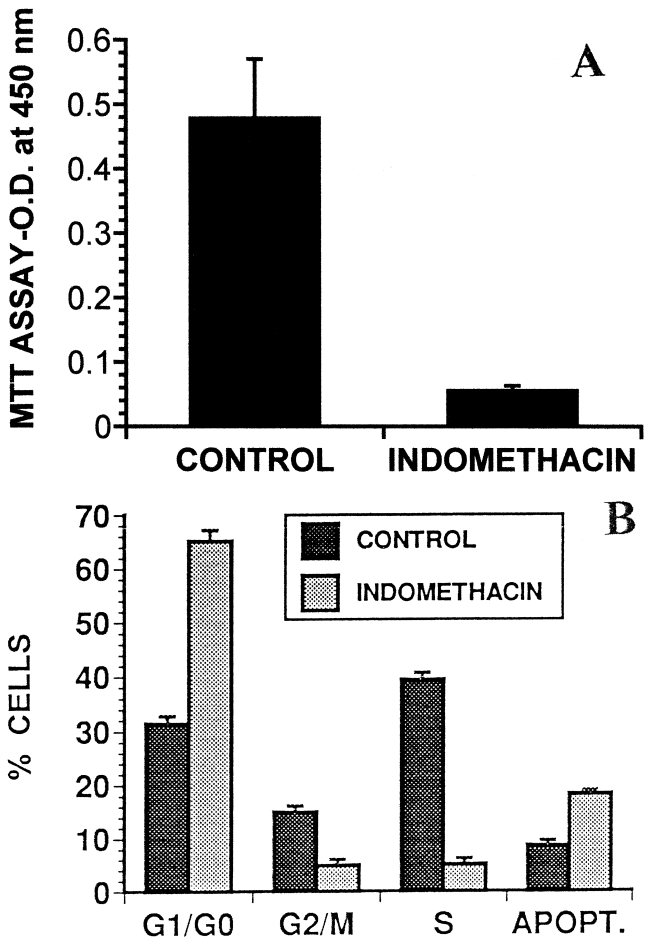

Fig. 4. Low dose indomethacin inhibits cell viability *in vivo* by causing an antiproliferative shift in cell cycle progression. (a) Mice were treated as described in the legend to Fig. 3. Tumors of approx. 8 mm in width (approx. 0.3–0.35 mL in volume) were obtained and the soft mass tumor cells isolated, incubated in 96-well plates for 24 hr and processed for MTT assay as described in Methods. Values are means ± SEM (3 mice/group; 12 wells/mouse). Values are significantly different (*t*-test, $P < 0.01$). (b) Mice were treated with indomethacin and inoculated with LL carcinoma cells as described in the legend to Fig. 1. For both control and indomethacin-treated groups, tumors were isolated when they reached approx. 0.3–0.35 mL in volume, and the soft tumor mass was resuspended in HBSS buffer. The upper cell suspension was diluted to 10^6 cells/mL and processed for cell sorter analysis as described in Methods. Values are means ± SEM of cells isolated from 6 mice in each group. Values of the two treatments are significantly different (*t*-test, $P < 0.01$).

signed to be similar to an antitumorigenic treatment course in humans following resection of the primary tumor. In mice subjected to the amputation-dependent natural metastases model, indomethacin at 2 mg/kg/day was found to be a highly potent inhibitor of metastatic tumor growth when given from the day of tumor inoculation (Fig. 5A). Indomethacin given only from the day of amputation was also effective in attenuating metastatic growth concurrent with prolonging the life of the treated mice from an average of 29 days postamputation for control animals to 56 days for the indomethacin-treated mice. We further tested the effect of indomethacin directly on metastatic growth in a model without a primary tumor, i.e. where the tumor cells are injected directly into the blood circulation. Indomethacin was also found to be highly effective in inhibiting lung metastatic growth in this direct metastases model (Fig. 5B).

Finally, we evaluated whether the antimetastatic effect of indomethacin was also accompanied by attenuation of tumor-associated cachexia. Using the direct metastases model, we found that whereas the control mice began to lose weight when lung tumor growth began to appear (days 10–17) and continued to lose weight thereafter, the indomethacin-treated mice continued to gain weight normally at least until the end of the experiment on day 24 (Fig. 5C). This group of mice did begin to develop metastases concurrently with cachexia, but only after an additional 2–3 weeks.

4. Discussion

There is a growing interest in understanding the mechanisms mediating the antiproliferative effects of NSAIDs and in determining whether these effects are linked to their cyclooxygenase-inhibiting properties or independent of them. Data obtained in *in vitro* studies with cultured cells show that NSAIDs inhibit cell proliferation and/or increase cellular necrosis/apoptosis, resulting in overall inhibition of cell growth [8–13]. These findings, however, show that these effects are seen only at extremely high NSAID doses, e.g. 600 μM indomethacin [11], 3–20 mM aspirin [11–13], or 200 μM naproxen [11], concentrations which are not generally found in the circulating blood or tissues of experimental animals or humans treated for inflammation-related diseases. A case in point is a recent study [13] in which aspirin and salicylate were found to be equipotent in inducing apoptosis and activation of caspases in cultured B-cell chronic lymphocytic leukemia cells when the drugs were given at concentrations of 5–10 mM. As pointed out by others [16], such plasma concentrations, if achieved, will be above the documented limit of toxicity in man [17], making their use in clinical situations unlikely. In a recent publication [18], indomethacin as well as the selective COX-2 inhibitor NS-398 were shown to exert inhibition of angiogenesis as assayed *in vitro* in cultured cells. This effect, however, was again seen at a relatively high concentration of 0.25–0.5 mM, which considerably exceeds the blood levels attained with these drugs when they are used effectively *in vivo*.

Our goal in the present study was to determine *in vivo* whether low dose indomethacin would exert antitumorigenic and/or antimetastatic effects and to investigate which phases of the cell cycle are affected by this treatment. We specifically contrasted the *in vitro* effects of indomethacin on cultured cells with those on tumor cells isolated from the *in vivo* growing tumor. Indomethacin provided to mice in the drinking water at 10 μg/mL (approx. 2 mg per kg/day) was found to be highly effective in delaying the growth of

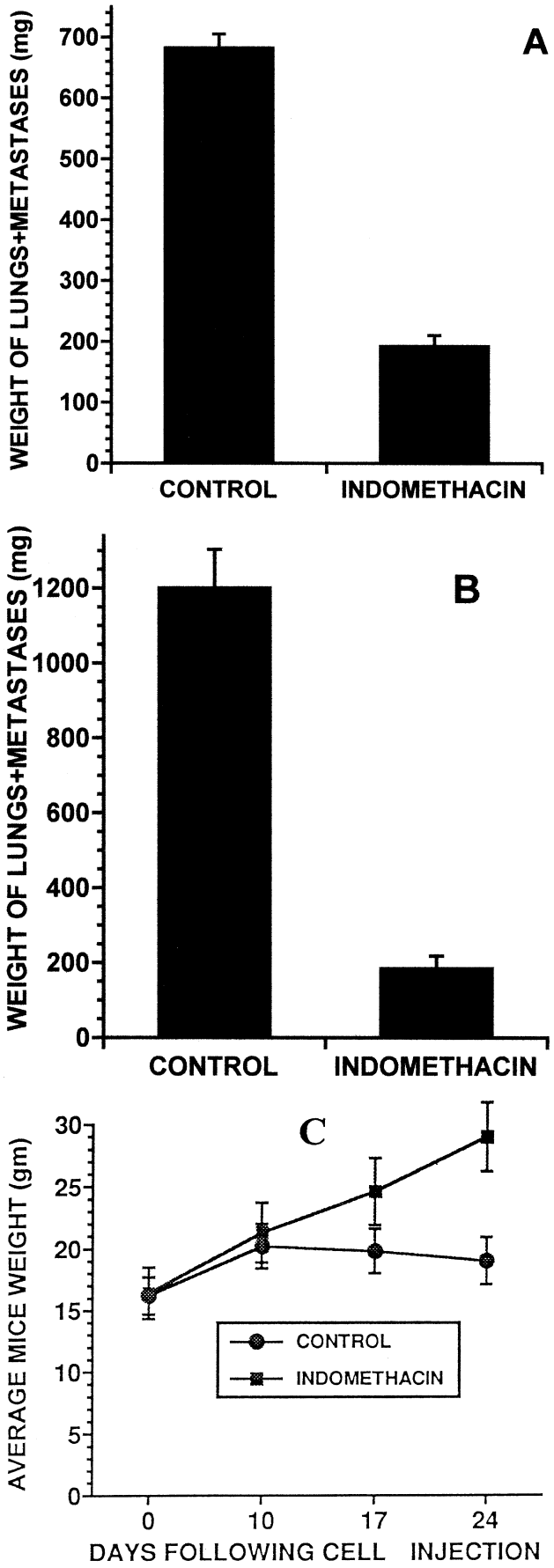

Fig. 5. Indomethacin inhibits growth of lung metastases and tumor-associated cachexia. Indomethacin was added to the drinking water (10 μg/mL) beginning one day before tumor resection (A) or before tumor cell injection into the tail vein (B) and continuing until termination of the experiment due to death from lung metastases in the control groups. Values are means ± SEM from 10 mice in each group and are significantly different between the two groups (t-test, $P < 0.01$). The average lung weight of uninjected mice was 160 ± 13 mg (N = 10). (C) Changes in body weight during the course of the experiments as described in (A). Values are means ± SEM of 10 mice per group and are significantly different (ANOVA for repeated measurements) at 17 ($P < 0.05$) and 24 days ($P < 0.01$).

both the primary tumor inoculate and of lung metastatic nodules. Mechanistically, we demonstrate here that indomethacin treatment *in vivo* causes acceleration of apoptosis as well as inhibition of cell proliferation. Significantly, indomethacin attenuated metastatic growth even when given only after primary tumor amputation, at which time micrometastases are already present in the lungs. All the indomethacin effects were seen at plasma concentrations of approx. 6 μg/mL, which bears direct relevance to the therapeutic blood levels of this drug in man. A recent report on the effects of COX inhibitors on tumor angiogenesis [19] demonstrated that indomethacin as well as the selective COX-2 inhibitor celecoxib inhibit tumor growth *in vivo* by blocking angiogenesis in the growing tumor [19]. Our results are in full agreement with these recent data and provide further support for the proposition that NSAIDs (except for sulindac, for which there is evidence otherwise; see Refs. 20–22) as well as COX-2 inhibitors exert their antitumorigenic and anti-angiogenic effects *in vivo* to a major extent through COX-dependent mechanisms.

Acknowledgments

This study was supported in part by the Ela Kodesz Institute for Research on Cancer Development and Prevention and by the N. Singer Foundation of Tel Aviv University.

References

[1] Hubbard NE, Chapkin RS, Erickson KL. Inhibition of growth and linoleate-enhanced metastasis of a transplantable mouse mammary tumor by indomethacin. Cancer Lett 1988;43:111–20.
[2] Shibata MA, Hasegawa R, Imaida K, Hagiwara A, Ogawa K, Hirose M, Ito N, Shirai T. Chemoprevention by dehydroepiandrosterone and indomethacin in a rat model of multiorgan carcinogenesis model. Cancer Res 1995;55:4870–4.
[3] Earnest DL, Hixson LJ, Alberts DS. Piroxicam and other cyclooxygenase inhibitors: potential for cancer chemoprevention. J Cell Biochem Suppl 1992;16I:156–66.
[4] Lundholm K, Gelin J, Hyltander A, Lonnroth C, Sandstrom R, Svaninger G, Korner U, Gulich M, Karrefors I, Norli B, *et al.* Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. Cancer Res 1994;54:5602–6.

[5] Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996;56:4566–9.
[6] Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998;58:409–12.
[7] Adolphe M, Deysson G, Lechat P. Action of some steroid and non-steroid anti-inflammatory agents on cell cycle: cytophotometric study of DNA content. Rev Eur Etud Clin Biol 1972;17:320–3.
[8] Hial V, De Mello MC, Horakova Z, Beaven MA. Antiproliferative activity of anti-inflammatory drugs in two mammalian cell culture lines. J Pharmacol Exp Ther 1977;202:446–54.
[9] Bayer BM, Beaven MA. Evidence that indomethacin reversibly inhibits cell growth in the G_1 phase of the cell cycle. Biochem Pharmacol 1978;28:441–3.
[10] Bayer BM, Kruth HS, Vaughan M, Beaven MA. Arrest of cultured cells in the G_1 phase of the cell cycle by indomethacin. J Pharmacol Exp Ther 1979;210:106–11.
[11] Shiff SJ, Koutsos MI, Qiao L, Rigas B. Nonsteroidal anti-inflammatory drugs inhibit the proliferation of colon adenocarcinoma cells: effects on cell cycle and apoptosis. Exp Cell Res 1996;222:179–88.
[12] Qiao L, Hanif R, Sphicas H, Shiff SJ, Rigas B. Effect of aspirin on induction of apoptosis in HT-29 human colon adenocarcinoma cells. Biochem Pharmacol 1998;55:53–64.
[13] Bellosillo B, Pique M, Barragan M, Castano E, Villamor N, Colomer D, Montserrat E, Pons G, Gil J. Aspirin and salicylate induce apoptosis and activation of caspases in B-cell chronic lymphocytic leukemia cells. Blood 1998;92:1406–14.
[14] Eisenbach L, Segal S, Feldman M. MHC imbalance and metastatic spread in Lewis Lung carcinoma clones. Int J Cancer 1983;32:113–20.
[15] O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis Lung carcinoma. Cell 1994;50:315–28.
[16] Guereno MT, Diez RA. Induction of apoptosis by salicylates in B-cell chronic lymphocytic leukemia. Blood 1999;93:1123–4.
[17] Insel PA. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman JG, Limbird LE, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. New York: McGraw-Hill, 1996. p. 617.
[18] Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh J, Tarnawski AS. Inhibition of angiogenesis by non-steroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat Med 1999;5:1418–23.
[19] Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner M, Edwards DA, Flickinger AG, Moore RG, Seibert K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60:1306–11.
[20] Piazza GA, Rahm AK, Finn TS, Fryer BH, Li H, Stoumen AL, Pamukcu R, Ahnen DJ. Apoptosis primarily accounts for the growth properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest and p53 induction. Cancer Res 1997;57:2453–9.
[21] Piazza GA, Alberts DS, Hixson LJ, Paranka NS, Li H, Finn T, Bogert C, Guillen JM, Brendel K, Gross PH, Sperl G, Ritchie J, Burt RW, Ellsworth L, Ahnen DJ, Pamukcu R. Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels. Cancer Res 1997;57:2909–15.
[22] Mahmoud NN, Boolbol SK, Dannerberg AJ, Mestre JR, Bilinski RT, Martucci C, Newmark HL, Chadburn A, Bartagnolli MM. The sulfide metabolite of sulindac prevents tumors and restores enterocyte apoptosis in a murine model of familial adenomatous polyposis. Carcinogenesis 1998;19:87–91.