Antiangiogenic Effects of the Quinoline-3-Carboxamide Linomide

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ABSTRACT

Linomide (N-phenylmethyl-1,2-dihydro-4-hydroxyl-1-methyl-2-oxoquinoline-3-carboxamide) has a reproducible in vivo antitumor effect against a series of both androgen responsive and independent Dunning R-3327 rat prostatic cancers. This antitumor effect of linomide is host mediated. One possible mechanism involving the host is that linomide has antiangiogenic activity. An indication that linomide treatment has antiangiogenic activity is the observation that prostatic cancers from linomide treated rats have more focal necrosis than sized matched tumors from untreated rats.

To directly test if linomide has antiangiogenic activity, a newly developed Matrigel based quantitative in vivo angiogenic assay was used. These experiments demonstrated that linomide has dose dependent, antiangiogenic effects in vivo in the rat. Additional studies demonstrated that due to its antiangiogenic activity, linomide treatment of rats bearing prostate cancers resulted in a more than 40% decrease in tumor blood flow. Blood flow to a variety of non-tumor bearing organs was not decreased suggesting that linomide selectively inhibits angiogenesis and does not induce loss of established blood vessels. Using as a model the response of human umbilical vein endothelial cells to linomide treatment in a variety of in vitro assays, linomide was demonstrated to have cytostatic but not cytotoxic effect on human umbilical vein endothelial cells at a medium concentration of ≥100 µg/ml. In addition, both endothelial cell chemotactic migration and invasion are steps in angiogenesis inhibited by linomide treatment.

INTRODUCTION

Linomide (N-phenylmethyl-1,2-dihydro-4-hydroxyl-1-methyl-2-oxoquinoline-3-carboxamide) (Fig. 1) has been demonstrated to have immunomodulating activity (1–5) and antitumor effects in various animal model systems. When given systemically to mice, linomide reduces primary and secondary tumor growth and metastases of murine B16 melanoma cells (6, 7) and Lewis lung carcinoma. It enhances the proliferation of mitogen stimulated T-cells (4) and provides a therapeutic treatment in some murine autoimmune syndromes (8). The antitumor effects of linomide in mice are believed to be mediated both by enhanced NK (6) activity and non-NK (i.e., macrophage) defense mechanisms (5, 6). When given systemically to rats, linomide enhances the delayed type hypersensitivity reaction to bacterial antigens (2), enhances mitogen stimulated proliferation of T-cells (1), and inhibits the growth of dimethylbenzanthracene induced mammary tumors (1). Recently it has been demonstrated that in vivo exposure of rat prostatic cancer cells to linomide, at doses equivalent to those producing therapeutic responses in vivo, is neither cytotoxic nor cytostatic (9). These data suggest that the anti-prostatic cancer effects of linomide require the involvement of the host. These studies further demonstrated that in the rat, linomide treatment did not enhance either NK cell number and/or NK cell induced cytotoxicity (9). In addition, treatment of the rats with antiasialo GM1, which suppressed NK cytotoxicity did not prevent the anti-prostate cancer effects of linomide (9). Likewise, the antitumor effects of linomide were also evident in nude rats. Thus, effects in addition to immune mechanism appear to be involved in the anti-prostatic cancer effect of linomide in the rat.

One possible nonimmune host mechanism which is consistent with the previous data is that linomide might have antiangiogenic activity in the rat. Also consistent with this possibility is the observation that histological sections of rat prostatic cancers from linomide treated rats have more focal necrosis than sized matched tumors from untreated hosts (9). Since focal necrosis is usually due to a limited tumor blood supply, these observations suggest that linomide treatment might have an inhibitory effect upon the angiogenesis occurring in vivo within developing prostatic cancers.

Angiogenesis, the formation of new blood vessels, is a complex process involving endothelial cell invasion, adhesion, chemotactic migration, proliferation, differentiation into tube-like structures, and the production of a basement membrane matrix around the vessel (10, 11). Angiogenesis occurs as a physiological process (e.g., embryonic angiogenesis, response to ovulation) as well as in some pathological processes, such as wound healing, chronic inflammation, certain immune responses (12), solid tumor growth (13), and other disease processes. In solid malignancies, angiogenesis is necessary for their continuous growth (14). The inability of solid tumors to grow to a clinically significant size or to metastasize in the absence of the successful induction of angiogenesis has prompted interest in the identification of compounds that block angiogenesis. Folkman (15) has hypothesized that the inhibition of angiogenesis might be a rational therapeutic approach to the control of tumor growth.

In the present study, the effects of linomide on angiogenesis were evaluated in several in vivo and in vitro systems. The aim of this work was to determine if linomide has antiangiogenic ability, and, if it does, to determine whether this is the basis of its in vivo antitumor effects.

MATERIALS AND METHODS

Reagents. Linomide is the registered trademark for roquinimex (Kabi Pharmacia Therapeutics, Helsingborg, Sweden). Linomide was synthesized at Kabi Pharmacia Therapeutics.

Matrigel, an extract of mouse basement membrane proteins, consisting predominantly of laminin, collagen IV, heparan sulfate proteoglycan, and nidogen/entactin, was prepared as a sterile solution as described by Kleinman et al. (16).

In Vivo Angiogenesis Assays. To quantitate the antiangiogenic properties of linomide, the method of Passaniti et al. (17) was used. A solution of basement membrane proteins (i.e., Matrigel), 10 mg/ml, was mixed with 1.0 mg/ml of basic fibroblast growth factor (R&D Systems, Minneapolis, MN) and 64 units/ml of heparin (Sigma Chemical Co., St. Louis, MO). The mixture (~1 ml) was allowed to form a soft gel and was then injected s.c. into inbred male
Copenhagen rats (Harlan Sprague-Dawley, Indianapolis, IN). Injections were performed bilaterally in each rat near the abdominal midline using a 21-gauge needle. Rats were either untreated (i.e., controls) or divided into 3 groups each of which was treated for 1 week with daily i.p. injections of either 1, 10, or 100 mg/kg/dose of linomide. Linomide treatment was continued for 8 days after Matrigel injection, when the 4 animals in each group were killed. The Matrigel pellet from each inoculation site was removed and weighed, and one-half was immediately frozen to be used for the hemoglobin assay and one-half was fixed in 10% buffered formalin for histological quantitation. Angiogenesis was quantitated by two methods.

In the first method, the hemoglobin present in the vessels within the Matrigel was measured by the Drabkin method (18) using the Drabkin reagent kit 525 (Sigma). These results were expressed as mg hemoglobin/g Matrigel pellet.

In the second method, using the histological sections, 10-20 fields were selected by the random systematic sampling technique (19) and the mean area of blood vessels/40 × magnification was determined by image analysis as described by Passaniti et al. (17). Briefly, the formalin fixed Matrigel was paraffin embedded and the sections were sectioned. Paraffin embedded tissue sections were deparaffinized, hydrated, and placed into 3% hydrogen peroxide to inactivate endogenous peroxidases. After rinsing in deionized water, the slides were enzymatically treated with 0.05% Protease Type XXVII (Sigma) in PBS at 37°C for 20 min. At the end of this treatment enzyme activity was abolished with 95% ethanol for 5 min. Slides were rinsed with PBS, and polyclonal rabbit anti-von Willebrand factor antibody (Dako, Carpintia, CA), diluted 1:200 in PBS, was applied to the slides for 1 h. After being rinsed in PBS biotinylated anti-rabbit IgG (Vector, Burlingame, CA), diluted 1:500 in PBS, were applied. Aminoethyl-carbazole solution containing hydrogen peroxide and DAB (Vector) was used to develop the samples. Slides were washed in tap water, dried, mounted in Crystal Mount (Biomeda Corp., Foster City, CA), and dried at 70°C for 20 min, and coverslips were attached with PermFont.

To measure the total area of neovessels, a computerized digitalizer, the Optomax image analysis system (Optomax, Hollis NH), was used. This system consists of a high sensitivity CCTV camera mounted on a Nikon Optiphot-2 microscope. The image is displayed on a color video monitor which is interfaced with a microprocessor. Histological slides stained with anti-von Willebrand factor antibody were examined by adjusting the color contrast to enhance the specifically stained blood vessels. The mean area ± SE, mm²/field at × 40 magnification, was calculated from 10-20 fields.

**Determination of Tumor Blood Flow.** The nonmetastatic, androgen independent, anaplastic Dunning R-3327 AT-2 rat prostate cancer was used for these tumor blood flow determinations. Developmental history and characteristics of this subline have been described previously (20). Inbred male Copenhagen rats (Harlan Sprague-Dawley) were matched for body weight and anesthetized with Metofane (Pittman Moore, Washington Crossing, NJ). Using sterile equipment, a small skin incision was made in the flank and a pocket was created into which ~8-10 mg of AT-2 tumor tissue was placed. The incision was closed using sterile autoclips. The animals were randomly divided into two groups: (a) one received linomide; and (b) the control group received saline as the vehicle. When tumors were 0.5-1.0 cm² in size (i.e., 10 days for the control group and 14 days for the linomide treated group), the tumor blood flow was determined using the radiolabeled microsphere technique (21). To do this, the rats were anesthetized with an i.p. injection of 50 mg/kg of sodium pentobarbital. The rats were tracheotomized and mechanically ventilated to maintain PaCO₂ at 35-40 mmHg and placed in a supine position on a thermostat regulated heating pad. The tail artery was canulated for monitoring the blood pressure. The right carotid artery was canulated with a polyethylene catheter (PE 10) which was threaded to the left ventricle of the heart. The left carotid artery was canulated with a polyethylene catheter (PE 50) which was threaded to the descending aorta and connected to a Harvard withdrawal syringe pump. Carbonized microspheres (16 ± 1.5 µm; DuPont-New England Nuclear) labeled with 153Gd were sonicated for 60 min and vortexed for 1 min before use. Reference blood samples were withdrawn from the descending aorta at a rate of 0.68 ml/min. After 15 s, approximately 1.8 × 10⁷ carbonized 153Gd-labeled microspheres in 0.3 ml of saline were injected through the right carotid catheter into the left ventricle of the heart and flushed with a 0.7 ml of Ringer's solution in 1 min. The reference blood withdrawal sampling was continued for 1.5 min. At the end of the protocol, the animals were killed with an i.v. injection of sodium pentobarbital and the tumors and variety of other organs were removed and weighted and their radioactivity was counted in a gamma radioactivity counter. Blood flow (BF) was calculated as:

\[
BF = \frac{Radioactivity/100 \text{ g of sample tissue}}{Radioactivity/100 \text{ g of reference blood sample}}
\]

**HUVEC Cell Cultures.** HUVEC cells were isolated from freshly delivered umbilical cords obtained from natural or caesarean births (22, 23). Briefly, the cells were detached with 0.1% collagenase in Hanks' balanced salt solution lacking calcium and magnesium, centrifuged, and resuspended twice and plated in a 100-mm plate in 5% CO₂, 95% O₂ at 37°C. At confluence, ~95% of the cells are endothelial, as determined by their distinctive morphology and the presence of factor VIII antigen on the cell surface. The cells were maintained in culture medium consisting of Medium 199 (Gibco, Grand Island, NY), 17% fetal bovine serum, 50 µg/ml endothelial mitogen (Biomedical Technologies, Stoughton, MA), 8000 units of heparin sodium, 100 units/ml penicillin/streptomycin, 50 µg/ml gentamicin, and 2 mm L-glutamine (Gibco) (i.e., HUVEC medium). Routinely, HUVEC cells from serial passage 4 to 7 were used in the following assays.

**HUVEC Cell Growth.** For growth determinations, HUVEC cells suspended in HUVEC medium containing 17% fetal bovine serum were seeded at \(2 \times 10^5\) cells/flask in T-25 flasks. After 24 h, the medium was removed and replaced with 5 ml fresh medium containing different concentrations of linomide (0, 10, 50, 100, 200, and 300 µM/ml). On day 2, 4, or 7, the medium was removed, plate trypsinized, combined with the removed medium, and centrifuged. The number of cells was counted in a cell counter. The number of viable cells was detected by trypan blue exclusion. All experimental measurements were run in triplicate and repeated four times.

**Cell Adhesion Assay.** To test the ability of linomide to effect the attachment of endothelial cells to either (a) type I collagen (Rat Tail Collagen, Collaborative Research, Bedford, MA); (b) fibronectin (Collaborative Research); (c) laminin (Collaborative Research); or (d) basement membrane Matrigel, cell adhesion assays were performed as described previously (24) with some modifications. Briefly, 24-well plates were coated with 5 µg of 1 of 4 different types of proteins or 25 µg of matrigel and dried overnight at room temperature in a sterile hood. Linomide in Medium 199 containing 2% fetal calf serum was added to each well at various concentrations (0, 10, 50, 100, 200, or 300 µg/ml). Then, \(5 \times 10^4\) HUVEC cells which were either nonpre-treated or pretreated for 24 or 48 h with medium containing the corresponding concentration of linomide were plated in individual wells and incubated in 5% CO₂ at 37°C. After a 2-h incubation period, the medium was removed, the plates were washed with PBS, and the wash was combined with the removed medium. This combined mixture was centrifuged and the numbers of cells was counted to determine the number of unattached cells. The washed plates were trypsinized and the number of cells was counted to determine the number of attached cells. The results are expressed as the percentage of the total cells which are attached. All experimental measurements were performed at least twice in duplicate or triplicate.

**Chemotactic Migration Assay.** Endothelial cell migration assays were performed using a modified Boyden chamber as described by Albini et al. (25). Briefly, 13-mm diameter polivinilpyrolidone-free polycarbonate filters with
an 8 µm pore size (Nuclepore Corp., Pleasanton, CA) were coated with 50 µl of 100 µg/ml of type I collagen and dried at room temperature in a hood. The coated filters were placed in Boyden chambers and 2 x 10⁵ HUVEC cells which were either nonpretreated or pretreated for 4 days with varying (i.e., 0–300 µg/ml) concentration of linomide added to the upper well in Medium 199 (Gibco), containing 0.1% bovine serum albumin and the corresponding concentration of linomide. HUVEC medium (described above) containing the corresponding concentration of linomide was placed in the lower well and the chambers were incubated at 37°C and 5% CO₂. After 5 h of incubation, the filters were removed, fixed with methanol, and stained as described by Fridman et al. (26). Nonmigrating cells on the upper surface of the filters were removed by wiping with cotton swabs. The cells that had migrated through the pores to various areas of the lower filter surface were trapped between the filter and glass slide and counted in 5 random X400 magnification fields. Each assay was performed at least twice in duplicate.

**Chemoinvasion Assay.** The invasive ability of HUVEC cells was assayed according to the method reported by Albini et al. (25). Briefly, the upper surface of the filters was precoated with the collagen as described above. Matrigel was applied to the upper surface of the filters (25 µg/filter) and dried in a hood. These filters were placed in Boyden chambers and 2 x 10⁵ HUVEC cells were added to the upper well in Medium 199 containing 0.1% bovine serum albumin and either 0, 10, 50, 100, 200, or 300 µg/ml of linomide. HUVEC medium contains the corresponding concentration of linomide was placed in the lower well and the chambers were incubated at 37°C and 5% CO₂. After 5 h, the filters were processed and quantitated as described for the chemotactic assay.

**Statistical Analysis.** Numerical values are expressed as the mean ± SE. Statistical analysis of significance were made by a one-way analysis of variance with the Kruskal-Wallis test. P ≥ 0.05 was considered statistically significant.

**RESULTS**

**In Vivo Effect of Linomide on Angiogenesis.** To assess the antiangiogenic properties of linomide in vivo, the Matrigel implant method of Passaniti et al. (17) was used as described above. Quantitation of angiogenesis in the implanted Matrigel was made by measuring the hemoglobin present in the vessels within dissected Matrigels and by image analysis of the area of blood vessels. For the hemoglobin assay, the results are expressed as mg of hemoglobin/g of Matrigel pellet. The hemoglobin content correlates with the extent of angiogenesis in this assay (17). Comparison of the hemoglobin values for the Matrigels from the untreated control animals at 8 days after implantation versus the values for the linomide treated animals (Table 1) demonstrates that at a dose of ≥ 10 mg/kg/day linomide has the ability to substantially inhibit angiogenesis in vivo (P < 0.05).

The mean area of blood vessels was determined by using the image analysis assay. Obtained results (Table 1) are consistent with the hemoglobin results and demonstrate that the mean blood vessel area within the Matrigel is substantially suppressed when the animals are treated daily with linomide at a dose of ≥10 mg/kg/day.

**Table 1** Dose-response effect of linomide on in vivo angiogenesis

<table>
<thead>
<tr>
<th>In vivo treatment for 8 days* (mg/kg/day)</th>
<th>Hemoglobin assay (mg of hemoglobin/g of Matrigel)</th>
<th>Image analysis (mean area µm² of blood vessels/400 field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>1,380 ± 158</td>
<td>31,035 ± 726</td>
</tr>
<tr>
<td>Linomide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1,329 ± 175 (4)</td>
<td>27,869 ± 358 (10)</td>
</tr>
<tr>
<td>10</td>
<td>285 ± 25 (79)</td>
<td>15,290 ± 973 (51)</td>
</tr>
<tr>
<td>100</td>
<td>449 ± 28 (68)</td>
<td>18,429 ± 599 (41)</td>
</tr>
</tbody>
</table>

* Eight samples/group.

**In Vivo Effect of Linomide on Tumor Induced Angiogenesis.** Based upon the Matrigel angiogenesis assay, it is clear that linomide treatment can inhibit neo-vessel formation in vivo in a dose dependent fashion. If linomide treatment induces a similar antiangiogenic response in prostatic cancers growing in vivo, this treatment should decrease blood flow to the tumor. To test this, Copenhagen rats were inoculated with Dunning AT-2 prostatic cancers. This tumor was chosen since it had previously been demonstrated to be highly responsive to 100 mg/kg/dose of linomide in vivo (9). Thus, one-half of the tumor bearing rats was treated daily with i.p. injections of 100 mg/kg/day of linomide, and one-half was given i.p. injections of saline. When tumors were ~5–1.0 cm³ in size, the tumor blood flow was determined using a radiolabeled microsphere technique (21). Using this method, it was demonstrated (Table 2) that 100 mg/kg/day of linomide substantially inhibited (~45% inhibition) the blood flow to AT-2 tumors in vivo. This inhibition was tumor specific since blood flow to other organs was not significantly affected (Table 2).

**In Vitro Effect of Linomide on HUVEC Cell Growth.** To test the possibility that linomide exerts its antiangiogenic activity by impairing endothelial cell proliferation, the chronic effect of linomide on the HUVEC cell growth was determined in vitro. These studies demonstrated that chronic exposure to linomide at concentrations ≥100 µg/ml (Fig. 2) slows the proliferation rate of HUVEC cells. To distinguish if this decrease in proliferation rate is due to a cytostatic or cytotoxic effects of linomide, HUVEC cells were grown until confluent, and then, chronic exposure to varying concentrations (i.e., 0–300 µg/ml) of linomide in the medium began. At 4 and 7 days of treatment, the number of viable cells was determined by trypan blue exclusion. The results demonstrated that linomide concentration of ≤300 µg/ml did not statistically decrease the number of viable HUVEC cells during 1 week of in vitro exposure.

**In Vitro Effects of Linomide on Attachment, Migration, and Invasion of HUVEC Cells.** Neither acute nor chronic (i.e., 48-h) exposure to different concentrations of linomide (0–300 µg/ml) inhibited the attachment of HUVEC cells to a variety of basement membrane constituents (i.e., matrigel, laminin, fibronectin, collagen type I). The results demonstrated that 75–85% of both nonpretreated or linomide pretreated HUVEC cells attached within 2 h regardless of the surface substrate or the concentration of linomide in the medium.

When HUVEC cells were cultured (i.e., only during assays) exposed to various concentrations of linomide from 0–300 µg/ml in a Boyden chemotactic chamber, their chemotactic migration was not inhibited (data not shown). However, decreased chemotactic response was produced when HUVEC cells were chronically pretreated for 4 days with linomide at a concentration of ≥100 µg/ml before being assayed (Table 3).

In contrast to the inability of acute or chronic linomide exposure to affect cellular attachment and acute exposure to inhibit chemotactic migration of HUVEC cells, acute (i.e., only during assays) linomide exposure decreases the ability of these cells to invade through reconstituted basement membrane in a dose dependent manner.

![Downloaded from cancerres.aacrjournals.org on July 13, 2017. © 1993 American Association for Cancer Research.](https://cancerres.aacrjournals.org/content/53/6/1385)
controls. It was observed, however, that prostatic cancers from did not demonstrate direct cytostatic or cytotoxic effect on prostatic cancer cell lines in vivo (9). When tested in vitro, however, linomide quinoline-3-carboxamide, linomide, has antitumor effects against a do not further decrease invasive abilities nor did the dose to linomide did not have a statistically significant difference versus controls. • Cells are pretreated for 4 days with indicated concentration of linomide before assays were used in Boyden chamber. • Numbers in parentheses, percentage of decrease in linomide treated versus untreated controls. • Statistically significant difference versus controls.

\[ \geq 100 \, \mu g/ml \] (Table 3), linomide was able to suppress the in vitro invasiveness of HUVEC cells. Chronic exposure of endothelial cells to linomide did not further decrease invasive abilities nor did the dose dependent inhibition change (data not shown).

**DISCUSSION**

Recent studies from our laboratory have demonstrated that the quinoline-3-carboxamide, linomide, has antitumor effects against a wide variety of both androgen dependent and independent rat prostatic cancer cell lines in vivo (9). When tested in vitro, however, linomide did not demonstrate direct cytostatic or cytotoxic effect on prostatic cancer cells. It was observed, however, that prostate cancers from linomide treated animals had more focal necrosis than identically sized tumors from untreated hosts. Since focal necrosis is usually due to a limited tumor blood supply, these results suggested that the anti-prostatic cancer effects of linomide could be indirectly mediated via an antiangiogenic response.

In the present studies, using the Matrigel angiogenesis assay, direct evidence was obtained that linomide has dose dependent antiangiogenic properties. Further results demonstrated that linomide treatment also affects tumor induced angiogenesis in vivo causing a decrease in prostatic cancer blood flow by more than 40% when given at a dose of 100 mg/kg/day. This dose regimen was chosen since previously it had been demonstrated to induce maximal anti-prostatic cancer effects in vivo against a broad range of rat prostate cancers (9). Blood flow to a variety of non-tumor bearing organs was not decreased in the rats treated with 100 mg/kg/day of linomide suggesting that linomide selectively inhibits neovascularization and does not induce the loss of established blood vessels. This could explain why daily treatment with 100 mg/kg/day of linomide does not induce generalized host toxicity even though this dose produces potent antiangiogenic effects. Rats have been given 125 mg/kg/day of linomide for more than 60 days with neither a decrease in body weight or food intake nor the development of any obvious outward signs of toxicity (9).

Angiogenesis is a complex process that involves a series of sequential steps, including the local degradation of the basement membrane of the parent vessel, pseudopodal protrusion and invasion of endothelial cells toward an angiogenic stimulus, sprout formation, DNA synthesis and mitosis of endothelial cells, sprout joining, and loop formation through which blood begins to flow (15). An angiogenesis inhibitor could inhibit any one or more of these steps and consequently inhibit new vascular formation. In order to clarify the mechanism by which linomide induces its antiangiogenic effects, the response of HUVEC to linomide treatment was used as a model system in a variety of in vitro assays. The ultimate goal of our studies is to develop a therapy for human prostatic cancers based upon an antiangiogenic mechanism; therefore, HUVEC cells were used as a model because of their human origin and because of their endothelial nature.

Since antiangiogenic activity may be exerted by impaired endothelial cell proliferation, we examined the dose-response effects of linomide on the growth of HUVEC cells. HUVEC cell growth in vitro was decreased in a dose and time dependent manner, but no effect on cell viability was recorded. Thus, linomide is cytostatic but not cytotoxic to endothelial cells. Antiangiogenic effects could be mediated also by an inhibition of the binding of the endothelial cells to different basement membrane components. In vitro studies demonstrated that neither acute or chronic exposure to linomide inhibited the attachment to HUVEC cells to a variety of cell attachment substrates. In contrast, linomide exposure inhibited chemotactic migration of HUVEC cells in vitro in a dose and time dependent manner. In addition, linomide exposure also decreased invasive ability of HUVEC cells in a dose dependent manner. In contrast to inhibition of chemotactic migration, inhibition of invasion of HUVEC cells did not require chronic (i.e., pretreatment) exposure to linomide. Further studies are in progress to resolve the mechanism by which acute linomide exposure of HUVEC cells inhibits their invasiveness. These studies are testing whether linomide is an inhibitor of any of the proteases associated with invasive ability (e.g., type IV collagenase, cathepsin B and D, and tissue plasminogen activator, etc.) Additional studies are examining the mechanism by which chronic, as opposed to acute, exposure to linomide modifies the chemotactic migration of HUVEC cells.

Regardless of its exact mechanisms, the fact that linomide has antiangiogenic activity in prostatic cancers in vivo is highly relevant. Prostate cancer is usually diagnosed in men over the age of 70 (27). Due to its late onset, approximately one of four men with clinically diagnosed prostatic cancer dies from the cancer and the remainder die of intercurrent disease (28). Any treatment which slows the growth of
prostatic cancer without unacceptable host toxicity would be beneficial in these elderly patients to suppress the progression of the disease to its lethal conclusion, even if not being curative. As demonstrated in animal models, linomide treatment is not curative for prostatic cancer when used as monotherapy and for the maximal therapeutic response it must be given continuously (i.e., chronically) (9). The decrease in tumor angiogenesis and the ensuing tumor blood flow decrease in linomide treated hosts results in an 50% suppression in prostate cancer growth regardless of whether the tumor is androgen responsive or androgen independent (9). This latter point is critical since initially prostatic cancers are usually highly responsive to androgen ablation therapy. Androgen ablation therapy is, however, rarely curative (29). This failure is due to the nearly universal progression of prostatic cancer to an androgen independent state (30). Due to the low toxicity of linomide and to its androgen independent ability to inhibit tumor angiogenesis and thus suppress tumor growth, linomide could be useful in the treatment of both locally advanced and metastatic prostatic cancer. Presently, the optimal management for either locally advanced or metastatic prostatic cancer is debated (e.g., immediate versus delayed androgen ablation therapy). Due to quality of life issues concerning immediate androgen ablation therapy, patients with locally advanced disease (i.e., clinical stage C) are often allowed to go untreated until the time when definitive metastasis are detectable and clinical problems develop (e.g., pain). In addition, even if definitive metastasis is detectable, such metastatic (i.e., clinical stage D) patients are usually not treated until clinical symptoms develop. Due to its androgen independent growth suppressive ability, an alternative to such delayed therapy could be to initiate chronic linomide treatment to such asymptomatic C and D stage patients to inhibit disease progression. Presently, the ability of long term (i.e., chronic) linomide treatment to suppress the progression of locally advanced and metastatic prostatic cancers in rats, as well as the host toxicity to such chronic linomide treatment, is being tested.

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