Inhibition of Tumor Growth and Metastasis of Rodent Tumors by the Angiogenesis Inhibitor O-(Chloroacetyl-carbamoyl)fumagillol (TNP-470; AGM-1470)

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ABSTRACT

The effect of the potent angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (TNP-470), a semisynthetic analogue of fumagillin, on tumor growth and metastasis was studied using rodent tumors. Injection of TNP-470 s.c. inhibited tumor growth in a dose-dependent manner, and the tumor sizes of B16BL6 melanoma, M5076 reticulum cell sarcoma, Lewis lung carcinoma, and Walker 256 carcinoma were maximally reduced to 16, 10, 17, and 4% of that in the respective control.

The activity of TNP-470 upon i.v. injection was slightly weaker than that following s.c. injection. This tendency was observed for all the tumors tested. Injection i.v. (infusion) of TNP-470 increased the life span of Walker 256 carcinoma-bearing rats by 18% over the control, while bolus i.v. injection increased the life span by only 47%.

TNP-470 reduced the number of pulmonary metastatic foci of i.v. inoculated B16BL6 melanoma in a dose-dependent manner, and the number of metastatic foci was reduced to 10% of that in the control by treatment with TNP-470 at 60 mg/kg, 3 times/week. The mean survival time of B16BL6 tumor-bearing mice treated with TNP-470 using this regimen was extended by 56% over that of control mice. TNP-470 at 10 mg/kg every day also reduced the number of metastatic foci of M5076 sarcoma in the liver after resection of the tumor from the primary site. Adriamycin at the same dose only slightly reduced the number of metastatic foci, even though TNP-470 and Adriamycin showed roughly equal inhibitory activity against M5076 sarcoma growth. TNP-470 extended the mean survival time of M5076 tumor-bearing mice by more than 100% over that of control mice at 30 mg/kg every 3 days, while Adriamycin extended mean survival times by maximally 20% at 10 mg/kg. These results show that the angiogenesis inhibitor TNP-470 has strong inhibitory activities against in vivo growth and metastasis of a wide variety of tumors.

INTRODUCTION

In normal adults, the vascular system is very stable. The turnover time for vascular endothelial cells in most tissues is measured in years (1). Angiogenesis only occurs in physiological states such as ovulation, menstruation, and the development of the placenta, or in pathological states such as diabetic retinopathy and solid tumor growth (2). Physiological angiogenesis is usually brief and tightly regulated, while indefinite growth of endothelial cells and abnormal structure of blood vessels are observed in pathological angiogenesis. It has been demonstrated that solid tumors are angiogenesis-dependent, that is, their growth beyond a certain size requires the formation of new blood vessels for the transport of nutrients and oxygen (3). In fact, several tumors have been reported to secrete angiogenic factors to elicit angiogenesis (4, 5). For these reasons, it is expected that inhibition of angiogenesis should provide powerful and selective therapy for a wide variety of cancers (6).

Tumor metastasis is a major cause of death in cancer patients and is one of the biggest obstacles to successful treatment. Tumor metastasis is a multistep process that includes angiogenesis (7–9). It has been suggested that the growth of micrometastases in target organs and tissues depends on angiogenesis (7). Recently, it was reported that there is a correlation between angiogenic properties and metastatic potential of human mammary carcinomas (10). Recent reports have also suggested that hormone-independent prostate and mammary carcinomas produce angiogenic factors such as basic fibroblast growth factor and pleiotrophin, which seem to be involved in metastasis (5, 11). These results suggest that specific inhibition of angiogenesis might keep micrometastasis dormant (2). We have reported that TNP-470,2 an analogue of fumagillin derived from Aspergillus fumigatus, selectively inhibits angiogenesis and the growth of some tumors (12, 13). In this study, the inhibitory activities of TNP-470 against in vivo growth and metastasis of rodent tumors were examined.

MATERIALS AND METHODS

Materials. TNP-470 (formulation for clinical trials) was synthesized at Takeda and used in all the in vivo experiments. Its structure has already been presented (12). TNP-470 was dissolved in 5% glucose solution. Reagents were obtained as follows. Eagle’s minimum essential medium, DMEM, and medium supplements were from GIBCO (Grand Island, NY), and FBS was from Whittaker (Walkersville, MD). Adriamycin (Adriacin) was purchased from Kyowa Hakko (Tokyo, Japan) and dissolved in 0.9% NaCl solution. All other materials were reagent grade. Intramedic polyethylene tubing PE50 (outer diameter, 0.965 mm) was obtained from Clay Adams ( Parsippany, NJ). Terufusion syringe pump ST-525 for i.v. infusion was purchased from Terumo (Tokyo, Japan).

Animals and Tumors. Specific pathogen-free female C57Bl/6 mice at 6 weeks of age and F344/Du rats at 3 weeks of age were purchased from Charles River Japan, Inc. (Yokohama, Japan). Animals were fed a commercial pellet diet (CE-2; CLEA Japan, Inc.) with water ad libitum.

B16BL6 melanoma (14, 15) was kindly provided by Dr. I. J. Fidler (M. D. Anderson Cancer Center, Houston, TX). M5076 reticulum cell sarcoma was obtained from the National Cancer Institute, NIH, Bethesda, MD, in 1983 through the courtesy of Dr. M. Saffran. Lewis lung carcinoma was kindly supplied by Dr. J. Folkman (The Children’s Hospital, Boston, MA). Walker 256 carcinoma was supplied by Dr. J. W. Wilks (The Upjohn Company, Kalamazoo, MI). M5076 and Walker 256 tumors were maintained in C57Bl/6 mice. C57Bl/6 CD4/Du rats, respectively. B16BL6 and Lewis lung cells were maintained as cultured cells. B16BL6 cells were cultured with Eagle’s minimum essential medium supplemented with 10% FBS and 1% nonessential amino acids as described elsewhere (15). The cells were passaged twice a week with 0.05% trypsin-0.02% EDTA. Those cells cultured for more than 3 months were not used for experiments because of possible changes in metastatic potential. Lewis lung cells were cultured with DMEM supplemented with 10% FBS.

Tumor Growth Assay. Tumor nodules of B16BL6 melanoma were collected 10 days after i.p. injection of 1 x 105 cells into C57Bl/6 mice, and 0.1 ml of 1:4 tumor homogenate in 0.9% NaCl solution was s.c. transplanted into C57Bl/6 mice on day 0. The tumors were weighed on day 13. Two million M5076 cells maintained in ascites fluid in C57Bl/6 mice were s.c. injected into C57Bl/6 mice on day 0, and the tumors were weighed on day 14. Walker 256 carcinoma homogenates were prepared by a method similar to that used for B16BL6 melanoma homogenates, and 0.1 ml of the homogenate in serum-free DMEM was s.c. transplanted to F344/Du rats on day 0. The tumors were weighed on day 18.

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2The abbreviations used are: TNP-470, O-(chloroacetyl-carbamoyl)fumagillol; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle medium.

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day 7. In the infusion experiment, polyethylene tubing was inserted into the jugular vein of a F344/Du rat under anesthesia, and the drug solution was infused with a Terufusion syringe pump. Treatment with TNP-470 was initiated on day 1 in all the experiments. The doses and dosing schedules were examined first and optimized for each tumor type so as to show maximum inhibitory effect on tumor growth and minimum toxicity.

**Experimental Metastasis Assay.** B16BL6 cells were harvested with phosphate-buffered saline containing 0.02% EDTA, and single cell suspensions were prepared with serum-free medium. Fifty thousand cells in 200 µl of the serum-free medium were injected into the lateral tail vein of a female C57Bl6 mouse at the age of 7 to 9 weeks. The lungs were excised and fixed 15 days after inoculation. Metastatic foci on the lung surface were counted under a dissecting microscope. TNP-470 was first given 6 h before inoculation. Thereafter, mice were treated 3 times a week for 2 weeks. In the experiment examining the effect of TNP-470 on survival, tumor-bearing mice were treated with TNP-470 until death.

**Spontaneous Metastasis Assay.** Two million cells of M5706 reticulum cell sarcoma maintained in ascites fluid in C57Bl/6 mice were i.d. transplanted into the right axillary region of the flank of C57Bl6 mice (day 0), and the primary tumor was resected on day 7 or 8. The liver was excised 20 days after inoculation, and metastatic foci on the surface were counted under a dissecting microscope. Two-fold-diluted Pelican black ink was i.v. injected before sacrifice to make the metastatic foci more conspicuous. In the survival experiment, TNP-470 was given until the host's death.

**Statistical Analysis.** Student's t-test was used in all statistical analysis.

**RESULTS**

**Tumor Growth Inhibition of B16BL6 Melanoma by TNP-470.** Injection s.c. of TNP-470 on days 1, 3, 6, 8, and 10 markedly inhibited the growth of B16BL6 melanoma in a dose-dependent manner (Table 1). When TNP-470 was given at a dose of 60 mg/kg, the tumor weight was reduced to 16% of that in the control group. Injection i.v. of TNP-470 showed activity slightly less potent than that upon s.c. injection. The tumor weight was reduced to 48% of that in the control group by i.v. injection of TNP-470 at a dose of 15 mg/kg every other day, while the tumor weight was reduced to 62% by the i.v. injection of TNP-470 at a dose of 45 mg/kg every 6 days (the same total dose). Decrease in body weight gain was observed at s.c. doses more than 30 mg/kg given on days 1, 3, 6, 8, and 10 (data not shown).

**Tumor Growth Inhibition of M5706 Sarcoma by TNP-470.** TNP-470 potently inhibited the growth of M5706 sarcoma in a dose-dependent manner upon s.c. and i.v. administration (Table 2). Injections s.c. and i.v. inhibited the tumor growth with similar potency. TNP-470 given s.c. at 5 mg/kg every day reduced the tumor weight to 10% of that in the control group. TNP-470 injected i.v. at 30 mg/kg every 3 days reduced the tumor weight to 15%. The body weight was mildly affected at a s.c. dose of 15 mg/kg every day, and an i.v. dose of 30 mg/kg every 2 days.

**Tumor Growth Inhibition of Lewis Lung Carcinoma by TNP-470.** Injection s.c. of TNP-470 on days 1, 3, 5, and 7 markedly inhibited the growth of Lewis lung carcinoma in a dose-dependent manner (Table 3). When TNP-470 was given at a dose of 60 mg/kg, the tumor weight was reduced to 17% of that in the control group. Injection i.v. of TNP-470 at 50 mg/kg on days 1, 3, 5, and 7 reduced the tumor weight to 46% of that in the control group (Table 4). TNP-470 at 100 mg/kg reduced the tumor weight to 4% of that in the control group. Injections s.c. and i.v. were more effective than the i.v. injection. When TNP-470 was injected over 30 min by i.v. infusion, the activity of TNP-470 was enhanced as compared with bolus i.v. injection. A longer infusion time (120 min) resulted in a slightly more potent inhibitory effect on the tumor growth. Some effect of TNP-470 on the body weight was observed at doses of more than 50 mg/kg. The effect of i.v. infusion of TNP-470 on body weight gain was milder than that of bolus i.v. injection of TNP-470 (data not shown).

**Tumor Growth Inhibition of Walker 256 Carcinoma by TNP-470.** Injection s.c. of TNP-470 on days 1, 3, and 5 potently inhibited the growth of Walker 256 carcinoma in a dose-dependent manner (Table 4). TNP-470 at 100 mg/kg reduced the tumor weight to 4% of that in the control group. Injection i.v. of TNP-470 at 50 mg/kg on days 1, 3, and 5 reduced the tumor weight to 46%. Injection s.c. was more effective than i.v. injection. When TNP-470 was injected over 30 min by i.v. infusion, the activity of TNP-470 was enhanced as compared with bolus i.v. injection. A longer infusion time (120 min) resulted in a slightly more potent inhibitory effect on the tumor growth. Some effect of TNP-470 on the body weight was observed at doses of more than 50 mg/kg. The effect of i.v. infusion of TNP-470 on body weight gain was milder than that of bolus i.v. injection of TNP-470 (data not shown).

Tumor-bearing rats in the control group died between day 11 and day 20, probably due to excessive bleeding from necrotic tumor lesion, and s.c. injection of TNP-470 on days 1, 3, and 5 extended the survival time (Table 5). Life span was increased by 93% over that of control animals at a dose of 50 mg/kg. A higher dose failed to extend the survival time further. Bolus i.v. injection of TNP-470 at a dose of 50 mg/kg until death extended the survival time by 47% over that of control animals, whereas i.v. infusion of TNP-470 extended the survival time by 183%.

**Inhibitory Effect of TNP-470 on Lung Colonization by B16BL6 Melanoma.** TNP-470 reduced the number of pulmonary metastatic foci of B16BL6 melanoma in a dose-dependent manner (Table 6). The effect was maximal at 60 mg/kg, and the weight loss was observed at 100 mg/kg (data not shown). When TNP-470 was s.c. injected at 60 mg/kg, the tumor weight was reduced to 17% of that in the control group. Injection i.v. of TNP-470 at 30 mg/kg on days 1, 3, 5, and 7 reduced the tumor weight to 46% of that in the control group.
Table 4 Tumor growth inhibition of Walker 256 carcinoma by TNP-470

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>No. of rats</th>
<th>Tumor growth inhibition (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>s.c.</td>
<td>12</td>
<td>241.5 ± 120</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>TNP-470</td>
<td>s.c.</td>
<td>25</td>
<td>1003 ± 289</td>
<td>42</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>50</td>
<td>370 ± 135</td>
<td>15</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>100</td>
<td>108 ± 44</td>
<td>4</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

Experiment 2

| Control| i.v.  | 7           | 2406 ± 503  | 100                        |         |
| TNP-470| i.v.  | 50          | 1107 ± 240  | 46                         | <0.001  |

Control Infusion

| TNP-470| Infusion | 8           | 2317 ± 657  | 100                        |         |
|        | Infusion | 50          | 680 ± 352   | 29                         | >0.001  |
| Control| Infusion | 7           | 2078 ± 876  | 100                        | >0.001  |
|        | Infusion | 50          | 387 ± 159   | 19                         | >0.001  |

* Mean ± SD.

** TNP-470 was injected by i.v. infusion (30 min).

** TNP-470 was injected by i.v. infusion (120 min).

Table 5 Effect of TNP-470 on the survival time of F344 rats bearing Walker 256 carcinoma

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>No. of rats</th>
<th>Survival time (days)</th>
<th>Increase in life span (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>s.c.</td>
<td>12</td>
<td>11.5 ± 4.8*</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNP-470</td>
<td>s.c.</td>
<td>25</td>
<td>12.7 ± 3.4</td>
<td>10.4*</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>50</td>
<td>22.2 ± 4.9</td>
<td>93.0</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>100</td>
<td>19.2 ± 3.4</td>
<td>67.0</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Experiment 2

| Control| i.v.  | 6           | 15.5 ± 4.6  | 100                  |                          |         |
| TNP-470| i.v.  | 50          | 22.8 ± 8.7  | 47.1                 |                          |         |
|        | Infusion | 4           | 43.8 ± 9.0  | 182.6                |                          | <0.001  |

* Mean ± SD.

** Treated on days 1, 3, and 5.

*** Percent increase in life span = (mean survival time of treated/mean survival time of controls) × 100 ± 100.

**** Treated on days 1, 3, 5, 7, 9, 11, 14, 18, 21, 24, and 27.

** 120 min.

Table 6 Effect of TNP-470 on experimental metastasis of B16BL6 melanoma

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of mice</th>
<th>No. of metastatic foci in the lung</th>
<th>T/C(%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>222 ± 51</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>TNP-470</td>
<td>30a</td>
<td>120 ± 47</td>
<td>54</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60a</td>
<td>22 ± 12</td>
<td>10</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>TNP-470</td>
<td>60a</td>
<td>9 ± 6</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a TNP-470 was administered s.c. 3 times/week.

b TNP-470 was administered s.c. once a week.

mg/kg 3 times a week and once a week, the number of pulmonary metastatic foci was reduced to 13 and 19%, respectively. TNP-470 also reduced the size of the metastatic foci (data not shown). Treatment of tumor-inoculated mice with TNP-470 at 30 and 60 mg/kg, 3 times a week, extended the mean survival time by 24% (P < 0.001) and 56% (P < 0.001) over that of control animals, respectively (Fig. 1). Autopsy of some mice on death revealed that the tumor filled the thoracic cavity. When TNP-470 was first given 2 days after inoculation and injected 3 times a week thereafter, similar results were obtained (data not shown). TNP-470 mildly affected body weight gain at a s.c. dose of 60 mg/kg, 3 times/week (data not shown).

Inhibitory Effect of TNP-470 on Liver Colonization by M5076 Sarcoma. In control mice whose primary tumors were resected 8 days after i.d. inoculation, approximately 400 metastatic foci of M5076 sarcoma were observed evenly distributed over the lobes of the liver on day 20 (data not shown). TNP-470 potently reduced the number of metastatic foci in a dose-dependent manner (Table 7). The metastasis to the liver was almost completely inhibited by the s.c. injection of TNP-470 at doses of more than 10 mg/kg every day or 30 mg/kg every 3 days. TNP-470 also reduced the size of the metastatic foci. The intermittent s.c. injection of TNP-470 at 10 mg/kg every 3 days reduced the number of metastatic foci in the liver more effectively than the s.c. injection of TNP-470 at 3 mg/kg every day when the same total dose of TNP-470 was administered. Even the s.c. injection of TNP-470 at 30 mg/kg every 6 days reduced the number of metastatic foci to 2% of that seen in the control group. TNP-470 did not affect body weight gain at doses used in this experiment. In this assay system, the antimetastatic effect of Adriamycin was weak. The s.c. injection of Adriamycin at 10 mg/kg every 3 days did not significantly reduce the number of metastatic foci, while Adriamycin at this
TNP-470 reduced the number of metastatic foci to 4% of that in the control group. Decrease in body weight gain was more severe in Adriamycin-treated mice than TNP-470-treated mice. TNP-470 exerted the mean survival time of M5076 tumor-bearing mice at doses higher than 3 mg/kg every 3 days, the frequency of injection was varied among different tumor models. The effect of oral administration of TNP-470 was weaker than that of s.c. and i.v. injections (data not shown). The tumor growth of M5076 sarcoma and Walker 256 was more sensitive to TNP-470 than that of B16BL6 melanoma and Lewis lung carcinoma. The sensitivity of tumor growth inhibition by TNP-470 in vivo did not correlate with the sensitivity in vitro to TNP-470 treatment (in preparation). This is compatible with the concept that tumor growth inhibition by TNP-470 is not exerted by direct growth inhibition but by angiogenesis inhibition. The following observations also support this. TNP-470 exhibited potent antiangiogenic activity in several assay systems (12, 13), and vascular endothelial cells including human umbilical vein endothelial (HUVE) cells showed the highest sensitivity to TNP-470 among the cell types, including tumor cells whose growth was inhibited in vivo in this study. TNP-470 injected s.c. does not reach the serum concentration (>3 μg/ml) to exert cytotoxicity against tumor cells. Potency of TNP-470 in tumor growth inhibition is much higher than that of its parent compound, fumagillin. Cytotoxicity of TNP-470 is essentially same with that of fumagillin, while antiangiogenic action of TNP-470 is more potent than that of fumagillin (12, 13). Thus, potency of tumor growth inhibition by these agents correlates well with their potency of antiangiogenic action. However, the possibility that the antiproliferative action of TNP-470 partly contributes to its inhibitory action against tumor growth can not dose inhibited the growth of M5076 sarcoma at the primary site as potently as TNP-470 at 10 mg/kg every 3 days (Table 8). At this dose, TNP-470 reduced the number of metastatic foci to 4% of that in the control group. Decrease in body weight gain was more severe in Adriamycin-treated mice than TNP-470-treated mice. TNP-470 extended the mean survival time of M5076 tumor-bearing mice at doses of more than 3 mg/kg with treatment every 3 days (Fig. 2). The mean survival time was extended by more than 100% over that of control mice at a s.c. dose of 30 mg/kg every 3 days. Adriamycin extended the mean survival time by 20% at a s.c. dose of 10 mg/kg given on days 8, 11, 14, 17, 20, 26, and 32. Adriamycin failed to extend the mean survival time when given at a dose of 3 mg/kg every 3 days or 30 mg/kg on days 8, 11, and 14. When Adriamycin was given at doses higher than 3 mg/kg every 3 days, the frequency of injection was reduced because of severe weight loss.

**DISCUSSION**

There is accumulating evidence indicating that solid tumor growth is generally angiogenesis-dependent (2, 6). Therefore, specific angiogenesis inhibitors should have inhibitory activity against the in vivo growth of a wide range of tumors. The angiogenesis inhibitor TNP-470 has been reported to have inhibitory activity against tumor growth in some mouse systems (12, 13), but the activity was not thoroughly examined. In this study, we described detailed examination of inhibitory activities of TNP-470 against in vivo growth and metastasis in several rodent tumor models.

TNP-470 strongly inhibited the growth of all the tumors tested. By optimizing the dosing regimen and the formulation, TNP-470 inhibited the tumor growth much more potently than the activity reported previously (12). The sensitivity to growth inhibition by TNP-470, however, varied among different tumor models. The effect of oral administration of TNP-470 was weaker than that of s.c. and i.v. injections (data not shown). The tumor growth of M5076 sarcoma and Walker 256 was more sensitive to TNP-470 than that of B16BL6 melanoma and Lewis lung carcinoma. The sensitivity of tumor growth in vivo did not correlate with the sensitivity in vitro to TNP-470 treatment (in preparation). This is compatible with the concept that tumor growth inhibition by TNP-470 is not exerted by direct growth inhibition but by angiogenesis inhibition. The following observations also support this. TNP-470 exhibited potent antiangiogenic activity in several assay systems (12, 13), and vascular endothelial cells including human umbilical vein endothelial (HUVE) cells showed the highest sensitivity to TNP-470 among the cell types, including tumor cells whose growth was inhibited in vivo in this study. TNP-470 injected s.c. does not reach the serum concentration (>3 μg/ml) to exert cytotoxicity against tumor cells. Potency of TNP-470 in tumor growth inhibition is much higher than that of its parent compound, fumagillin. Cytotoxicity of TNP-470 is essentially same with that of fumagillin, while antiangiogenic action of TNP-470 is more potent than that of fumagillin (12, 13). Thus, potency of tumor growth inhibition by these agents correlates well with their potency of antiangiogenic action. However, the possibility that the antiproliferative action of TNP-470 partly contributes to its inhibitory action against tumor growth can not
be fully excluded. The high sensitivity of *in vivo* growth of M5076 sarcoma and Walker 256 carcinoma to TNP-470 might indicate the high dependency of these tumors on angiogenesis. M5076 sarcoma has been reported to be sensitive to other angiogenesis inhibitors (16). Many angiogenesis inhibitors have been reported to date, but tumor growth inhibition of only a few has been reported (16–21). TNP-470 seems to be one of the compounds that has most potent antiangiogenic activity and inhibitory activity against tumor growth among these types of inhibitors.

Angiogenesis inhibitors have also been reported to have efficient metastasis inhibitors based on the concept that tumors require newly formed blood vessels for their growth at both primary and secondary sites (7) and dissemination (2, 22). Potent antitumoral action was demonstrated with TNP-470 in 2 metastasis models. Experimental metastasis is a model for the latter half of the metastasis process, consisting of tumor cell adhesion to capillary endothelial cells, extravasation, and growth of micrometastasis. In this assay system using B16BL6 cells (13, 15), TNP-470 reduced both the number and the size of the metastatic foci in the lungs. M5076 reticulum cell sarcoma spontaneously metastasizes to the liver from primary sites via a multistep process that is thought to be common to solid tumors. The antitumoral activity of TNP-470 was more potent against M5076 sarcoma than against B16BL6 melanoma, as is also the case for the inhibitory activity against tumor growth.

TNP-470 extended the survival time of B16BL6 and M5076 tumors-bearing mice. Compounds reported to extend the survival time of B16BL6 and M5076 tumor-bearing mice do not exceed the potency of TNP-470 (17, 23–25). These results also suggest the low toxicity of TNP-470.

Inhibitory activities of TNP-470 and a chemotherapeutic agent, Adriamycin, against tumor growth and metastasis were compared in M5076 sarcoma models. The potency of these drugs for inhibition of primary tumor growth and metastases was not parallel. Adriamycin did not exhibit a significant antitumoral action at the doses causing tumor growth inhibition. In other words, Adriamycin is more effective on primary tumors than on secondary ones. This phenomenon could be explained, at least in part, by the difference in microenvironment and the acquisition of resistance to chemotherapeutic agents (26, 27). On the other hand, the target of angiogenesis inhibitors is normal endothelial cells, which do not easily develop resistance. This shows that the growth of metastatic foci and that of primary tumors exhibit different degrees of sensitivity to Adriamycin, and suggests that angiogenesis inhibitors would be more suitable as antitumoral agents than chemotherapeutic agents such as Adriamycin.

Several compounds have been reported to inhibit blood-borne metastasis. Among them are compounds that inhibit tumor cell adhesion, type IV collagenase activity, or coagulation (28–32). Since 60% of cancer patients with newly diagnosed solid tumors have clinically detectable or occult metastasis, antitumoral compounds that interfere with only the dissemination and the settlement of tumor cells might be of limited value for clinical use. Angiogenesis inhibitors also inhibit the late phase of metastasis and growth of micrometastases after settlement, and keep them dormant (2). Therefore, angiogenesis inhibitors have some advantages for development as antitumoral drugs.

For clinical use, the toxicity of antitumoral drugs should be low because of the possibility of a prolonged treatment period. The toxicity of specific inhibitors of endothelial cell growth is expected to be low since the turnover time of endothelial cells in normal tissues is extremely long, and angiogenesis occurs in limited physiological states such as follicle development, corpus luteum formation, and wound healing (2). TNP-470 had almost no effect on body weight gain of M5076- and Lewis lung-bearing mice at doses used in this study. TNP-470 had some effect on body weight gain of B16BL6-bearing mice and Walker 256-bearing rats at higher doses used in this study. Thus, the effect of TNP-470 on body weight gain seems to depend on inoculated tumors. When the treatment was discontinued, the rate of body weight gain rapidly returned to control level (data not shown). No toxic death was observed even at the highest dose administered in this study. Adriamycin more severely affected body weight gain of M5076 tumor-bearing mice than TNP-470 at doses that exerted comparable inhibitory activity against tumor growth. The effect of TNP-470 on body weight gain of Walker 256-bearing rats was found to be reduced by i.v. infusion. In conclusion, the angiogenesis inhibitor TNP-470 seems to be a promising candidate for development as an antitumor and antitumoral agent.

**REFERENCES**

INHIBITION OF TUMOR GROWTH AND METASTASIS BY TNP-470


Inhibition of Tumor Growth and Metastasis of Rodent Tumors by the Angiogenesis Inhibitor O-(Chloroacetyl-carbamoyl)fumagillol (TNP-470; AGM-1470)

Masuo Yamaoka, Toshihiro Yamamoto, Tsuneo Masaki, et al.