Advances in Brief

Antiangiogenic Agents Potentiate Cytotoxic Cancer Therapies against Primary and Metastatic Disease

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Abstract

The formation of a blood supply (angiogenesis) is critical to the growth of solid tumors. The naturally occurring steroid tetrahydrocortisol, the synthetic cycloextrim derivative \( \beta \)-cycloextrin tetradeacasulfate, and the tetracycline derivative minocycline have antiangiogenic activity. Tetrahydrocortisol and \( \beta \)-cycloextrin tetradeacasulfate in a 1:1 molar ratio by continuous infusion over 14 days and minocycline administered i.p. over 14 days from day 4 to day 18 postimplantation of the Lewis lung carcinoma significantly increased the growth delay of the primary tumor after treatment with cis-diaminedichloroplatinum(II), melphalan, cyclophosphamide, Adriamycin, bleomycin, and radiation therapy administered in standard regimens. Addition of the antiangiogenic agents to treatment with the cytotoxic therapies not only reduced the number of lung metastases formed from the primary tumor but also reduced the number of large metastases. Five of 12 animals treated with the antiangiogenic modulators and cyclophosphamide were long-term survivors (>120 days). Thus, antiangiogenic therapies can potentiate the efficacy of standard anticancer therapies.

Introduction

In order to enlarge and invade locally or distally, \( i.e., \) metastasize, a tumor must cause the breakdown and restructuring of the extracellular matrix including both basement membrane and interstitial matrices (1–5). It has been recognized for many years that the formation of a blood supply (angiogenesis) is critical to the growth of both normal and malignant tissues (6–9). For over 20 years cancer researchers have been searching for agents which could inhibit the processes of malignant cell invasion and growth in normal tissues. The angiostatic activity of several steroids was discovered in 1983 (10–12). Angiostatic steroids appear to induce basement membrane dissolution as part of their antiangiogenic action (10, 11). Of the naturally occurring angiostatic steroids, tetrahydrocortisol was identified as the most potent. Tetrahydrocortisol lacks the glucocorticoid and mineralocorticoid activity of other members of that steroid family. Folkman et al. (12) reported that \( \beta \)-cycloextrin tetradeacasulfate in combination with hydrocortisone was 100 to 1000 times more effective than heparin in combination with hydrocortisone in inhibiting capillary formation in the chick chorioallantoic membrane assay and in preventing neovascularization induced by endotoxin in the rabbit cornea (13–15).

Serine proteases and metalloproteases are involved in the degradation of the extracellular matrix during malignant tumor growth and invasion (16, 17). Type IV collagen is the main component of the tight structure of the basement membrane. Activity of type IV collagenase has been associated with the metastatic phenotype. Relatively high concentrations of corti

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3 \( \beta \)-Cycloextrin tetradeacasulfate was prepared in our laboratory by an improved method compared with those previously published. Briefly, a solution of sulfur trioxide pyridine complex (6.82 g; 0.042 mol; Aldrich Chemical Co., Milwaukee, WI) in anhydrous pyridine (12 ml) was heated on an oil bath in a 3-necked flask equipped with a condenser topped by a drying tube (calcium chloride) and a thermometer. Magnetic stirring was maintained at 80–85°C for 20 min to produce a pale yellow liquid. To this solution, \( \beta \)-cyclodextrin (1.135 g, 0.001 mol; Sigma Chemical Co., St. Louis, MO) was added rapidly with constant stirring. The reaction mixture was stirred at 80–85°C for 6 h and then allowed to stand at room temperature for 36–40 h to form a dark brown semisolid which was treated with methanol until the filtrate was colorless, it was air-dried and redissolved in 30% sodium acetate solution (1.933 g of sodium acetate:3H2O in 6.5 ml of water) and water (7 ml) to convert the pyridine salt into the sodium salt. This solution was gradually added into 100 ml of absolute ethanol and stirred for 1 h to allow precipitation of the \( \beta \)-cyclodextrin tetradeacasulfate sodium salt which was collected by filtration and washed with alcohol and then air-dried. The crude sodium salt was redissolved in 30% sodium solution (4 ml) and water (7 ml) and precipitated from alcohol (120 ml) 3 times as described above. The purified material was dried under vacuum over phosphoric pentoxide at 78°C/100 torr in a drying pistol for 4 h to give a white amorphous powder (2.35 g; 83.6% of yield).

4 \( \text{Ca}_4\text{H}_{25}\text{O}_{7}\text{Na}_{14} \times 14\text{H}_2\text{O} \) (M, 2815.7761)

5 \( \text{Calcified}: \text{C} 17.91, \text{H} 3.06, \text{S} 15.94 \)

Found: \( \text{C} 18.12, \text{H} 2.94, \text{S} 15.95 \)

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cell implantation, cytotoxic therapy was initiated. To different groups (6 animals/group), cis-Diaminedichloroplatinum(II) (10 mg/kg), melphalan (10 mg/kg), and cyclophosphamide (150 mg/kg) were administered i.p. on day 7. In other animals cyclophosphamide (150 mg/kg) were administered on days 7, 9, and 11 post-tumor implant. In other groups radiation was delivered locally to the tumor-bearing limb as a single dose of 20 Gy on day 7 or 3-Gy fractions daily on days 7-11. Adriamycin (1.75 mg/kg) was administered i.p. on days 7-11. Bleomycin (10 mg/kg) was administered i.p. on days 6, 10, 13, and 16.

Results and Discussion

The antiangiogenic therapy (THC4 cis-Diaminedichloroplatinum, melphalan, and single and multiple doses of cyclophosphamide, respectively, where the fold increase represents the increase in tumor growth delay that occurs when the modulators are added to the chemotherapy as compared to the chemotherapy alone. Five of 12 of the animals treated with the three-modulator combination and multiple doses of cyclophosphamide were long-term survivors (>120 days). The tumor growth delays produced by single-dose and multiple-dose radiation therapy were increased about 2.2- and 2.8-fold, respectively, in the presence of the three-modulator combination. The increases into tumor growth delay observed with Adriamycin and bleomycin with the addition of the three modulators to the regimen were about 1.7- and 1.5-fold compared with the antitumor agents alone. Neither minocycline as a single modulator (30) nor THC/cis-Diaminedichloroplatinum/tetrahydrocortisol/tetrahydrocortisol from day 4 through day 18 post s.c.

Table 1 Growth delay of the Lewis lung produced by various anticancer treatments alone or with a combination of antiangiogenic modulators, β-cyclodextrin tetraeddrosulfate/tetrahydrocortisol, minocycline

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose a</th>
<th>Alone</th>
<th>+14(SO4) βCD/THC/minocycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>14(SO4)βCD/THC/minocycline</td>
<td>1 x 10 mg/kg</td>
<td>4.5 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>cis-Diaminedichloroplatinum (II)</td>
<td>1 x 10 mg/kg</td>
<td>2.7 ± 0.3</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1 x 10 mg/kg</td>
<td>7.2 ± 0.4</td>
<td>27.6 ± 2.8</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1 x 150 mg/kg</td>
<td>21.5 ± 1.7</td>
<td>48.8 ± 3.3</td>
</tr>
<tr>
<td>Radiation</td>
<td>1 x 20 Gy</td>
<td>6.2 ± 0.5</td>
<td>13.8 ± 1.3</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>5 x 3 Gy</td>
<td>4.4 ± 0.3</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>5 x 1.75 mg/kg</td>
<td>7.0 ± 0.6</td>
<td>12.0 ± 1.4</td>
</tr>
<tr>
<td>14(SO4)βCD/THC/minocycline</td>
<td>4 x 10 mg/kg</td>
<td>8.5 ± 0.6</td>
<td>13.0 ± 1.5</td>
</tr>
</tbody>
</table>

- a β-Cyclodextrin tetraeddrosulfate (1000 mg/kg) and tetrahydrocortisol (125 mg/kg) were administered in a 1:1 molar ratio by continuous infusion over 14 days in an Alzet osmotic pump from days 4–18 post-tumor implant. Minocycline (5 mg/kg) was administered i.p. on days 4–18 post-tumor implant. cis-Diaminedichloroplatinum (II) (10 mg/kg), melphalan (10 mg/kg), and cyclophosphamide (150 mg/kg) were administered i.p. on day 7 post-tumor implant. Cyclophosphamide (150 mg/kg) was also administered on days 7, 9, and 11 post-tumor implant. Radiation was delivered locally to the tumor-bearing limb as 20 Gy on day 7 or 3-Gy fractions daily on days 7–11. Adriamycin (1.75 mg/kg) was administered i.p. daily on days 7–11. Bleomycin (10 mg/kg) was administered i.p. on days 6, 10, 13, and 16.

b Tumor growth delay is the difference in days for treated tumors to reach 500 mm³ compared with untreated controls tumor. Untreated control tumors reach 500 mm³ in about 14 days. Mean ± SE of 15 animals.

c Significantly different from result for the corresponding treatment alone group by the Dunn multiple comparisons test, P < 0.0001.

d Four of 12 animals were long-term survivors (>120 days).

e Five of 12 animals were long-term survivors (>120 days).

f P < 0.001.

Table 2 Number of lung metastases on day 20 from s.c. Lewis lung tumors after various anticancer treatments alone or in combination with β-cyclodextrin tetraeddrosulfate/tetrahydrocortisol, minocycline

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose a</th>
<th>Alone</th>
<th>+14(SO4) βCD/THC/minocycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>14(SO4)βCD/THC</td>
<td>100 mg/kg/125 mg/kg over 14 days</td>
<td>14.5 (10)</td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>14 x 5 mg/kg</td>
<td>12</td>
<td>5 (0)</td>
</tr>
<tr>
<td>cis-Diaminedichloroplatinum</td>
<td>as above</td>
<td>13</td>
<td>6 (2.5)</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1 x 10 mg/kg</td>
<td>12</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1 x 150 mg/kg</td>
<td>8</td>
<td>5 (0)</td>
</tr>
<tr>
<td>Radiation</td>
<td>1 x 20 Gy</td>
<td>8</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>5 x 1.75 mg/kg</td>
<td>7</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>5 x 10 mg/kg</td>
<td>7</td>
<td>4 (2)</td>
</tr>
</tbody>
</table>

- a The schedules of drug administration were as shown in Table 1.
- b The number of external lung metastases on day 20 post-tumor implant as counted manually and scored as >3 mm³ in diameter. Data are the means from 6-12 pairs of lungs. Numbers in parentheses, number of large (vascularized) metastases.
tumor implantation had little effect on the total number of lung metastases; however, only 40–50% of the metastases were large compared with about 70% in the untreated control animals. The cytotoxic antitumor treatments reduced the number of lung metastases in many cases to about one-half the number observed in the untreated control animals.

The antiangiogenic treatment combination along with the cytotoxic therapies was more effective against metastatic disease. In most cases, the number of lung metastases was reduced to about 50% of the number observed with the cytotoxic therapy alone and the number of large metastases was 40–50% of those. The lowest number of large metastases was found in animals treated with cyclophosphamide and antiangiogenic agents; in fact with multiple doses of cyclophosphamide in combination with minocycline there were no large lung metastases present on day 20. Although minocycline (30) and THC/14-(SO4)2CD5 were somewhat effective as modulators of metastatic disease response, the three-modulator combination was more effective.

Although cytotoxic therapies often produce regression in common solid tumors, a cure is rarely attained. We have been searching for modulators to add to standard therapies, which, by virtue of their effects on the physiological, biological, or biochemical properties of the tumor, would increase the susceptibility of the tumor to cytotoxic treatment without increased toxicity to the host. Agents which inhibit in vitro angiogenesis and processes involved in restructuring of the extracellular matrix could possibly act as modulators of other therapies by inhibiting further growth or regrowth both of primary and metastatic disease and inhibiting further metastatic invasion. In the ideal situation angiostasis might lead to the death of those tumor cells most distal from established tumor vasculature and thereby could reduce the tumor burden of the host and result in tumor mass more easily permeated by chemotherapy agents and treated by radiation therapy. Mechanisms other than inhibition of angiogenesis may be involved in the modulation of these cancer therapies by THC/14(SO4)-βCD/minocycline; neither THC/14(SO4)βCD5 nor minocycline (30) enhance the cytotoxicity of anticancer drugs or radiation in vitro.

The pathways available for extracellular matrix breakdown and restructuring in vivo are highly redundant. It is unlikely that 100% blockade of any given enzyme can be achieved in vivo; however, partial inhibition of multiple pathways may achieve a significant biological effect. When the three antiangiogenic modulators were administered together marked improvements in the response of primary and metastatic disease to several of the cytotoxic therapies occurred. These results indicate that antiangiogenic therapies are compatible with and can be therapeutically important additions to the treatment of solid tumors.

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References

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