Experimental and Clinical Activity of Mitomycin C and cis-Diamminedichloroplatinum in Malignant Mesothelioma

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ABSTRACT

Little information is available regarding the effectiveness of chemotherapeutic agents in malignant mesothelioma. Human malignant mesothelioma specimens from two patients were successfully transplanted and maintained in homozygous nude mice. Screening of chemotherapeutic agents revealed that cisplatin was the most active single agent in one line, and mitomycin C in the other. The combination of mitomycin C and cisplatin, however, was the most effective regimen for both lines of xenografted human mesothelioma. Based on these results in nude mice, a clinical trial of mitomycin C and cisplatin was undertaken. Four of 12 patients showed objective response (one complete and three partial). These clinical results support the usefulness of the nude mouse model for malignant mesothelioma.

INTRODUCTION

Malignant mesothelioma is a disease of growing importance. As predicted (25), its incidence is increasing (17) as a result of the widespread use of asbestos, its major etiological agent. Results of various therapies for malignant mesothelioma have been generally poor (5, 8). Neither surgery nor radiotherapy is curative. Very little information is available regarding the activity of chemotherapeutic agents (4, 8).

The availability in our laboratory of 2 distinct cell lines of human malignant mesothelioma serially transplanted into nude mice (6) allowed us to study systematically the activity of chemotherapeutic agents alone and in combination in this model. One combination, mitomycin C and cisplatin, exhibited efficacy against both lines in the nude mouse system (7). Based on this research, a clinical trial of this combination was initiated in 12 consecutive patients with malignant mesothelioma.

MATERIALS AND METHODS

Nude Mouse Xenografts. Two lines of human malignant mesothelioma (designated BG and ES) have been serially transplanted s.c. since 1978 in nude mice, as previously described (6). For these experiments, 8- to 10-week-old homozygous female nude (nu/nu) mice were obtained from the Animal Genetics and Production Branch of the National Cancer Institute, Bethesda, MD. Filtered air and sterilized bedding, cages, food, and water were used. Serial transplantation of tumors was carried out by excision of tumors under light anesthesia by inhalation with methoxyflurane (Penthrane; Abbott Laboratories, North Chicago, IL). The tumor specimens were dissected under sterile conditions in a Petri dish containing Roswell Park Memorial Institute Medium 1640 (Grand Island Biological Co., Grand Island, NY) and trimmed of cutaneous, connective, and adipose tissues under a fiberglass tissue culture hood (Fisher Scientific Co., Pittsburgh, PA). Small tumor cubes measuring 1 to 2 mm were inserted in a trocar and transplanted s.c. into 4 sites/mouse in the right and left groin and axilla, or into one site/mouse in the right axilla.

Since single-cell suspensions were not used, the number of cells transplanted in these specimens can only be determined on histological sections. For that purpose, tumor specimens were fixed in 10% neutral formalin and embedded in paraffin after dehydration in alcohol. Five- to 6-μm sections were made and stained with hematoxylin and eosin. Histological sections were examined with a Nikon light microscope equipped with an ocular micrometer calibrated with a hemacytometer (American Optical Scientific Instruments, Buffalo, NY). The number of tumor cells per cu mm was determined by numerical analysis in 20 random fields at ×400 for each line BG and ES (9), after correction for section thickness, fixation shrinkage (2), and proportional tumor cell content. Under these conditions, the number of tumor cells per cu mm was [4.8 ± 0.18 (S.E.)] × 10⁶ for BG xenografts, and [3.3 ± 0.32] × 10⁶ for ES xenografts.

Mice were observed every weekday and weighed once weekly. Both lines BG and ES have retained their original histological, histochemical, and ultrastructural features despite serial transplantation (6, 30). Karyotypes have confirmed their human nature (6). Line BG is an epithelial malignant mesothelioma, and line ES is a mixed or biphasic, but predominantly epithelial malignant mesothelioma (6). After the growth of s.c. implants was established, cohorts of mice transplanted with the same tumor specimen (one or 4 s.c. implants/mouse) were randomized between chemotherapy (2 to 6 animals/group) and control with 0.9% NaCl solution (2 animals/group). All injections were given i.p.

Quantification of Antineoplastic Activity. Tumor volume was measured with calipers once or twice weekly, using the formula for a prolate ellipsoid

\[ V = \frac{\pi}{6} l W^2, \]

where \( L \) is the greatest length and \( W \) is the perpendicular width of the tumor corrected for skin thickness (12, 19) (Fig. 1). Overall treatment effect was measured using the mean ratio \( T/C \) (expressed as a percentage) of the tumor volumes between Days 35 to 49 after tumor implantations for treated animals (T) over the tumor volumes of corresponding controls (C) (12). A T/C ratio of 42% or less is considered to be an indication of activity (12). Life span was measured from the time of tumor implantation to death. Statistical comparison of means between treated animals and controls was carried out using Student’s t test, with modification for small numbers.

In addition, a method of quantification of antineoplastic effect was designed which did not assume a preconceived pattern of growth but which examined the efficacy of therapy as reflected in the shrinkage of tumor mass in each individual experiment. For individual tumors, regarded as independent, each tumor volume \( N(t) \) as a function of time \( t \) was fit by a polynomial spline technique (10, 23), and integrated analytically from the time of initiation of therapy at \( t_a \) to the time \( t_b \) of last volume measurement prior to death. The parameter \( A \), which can be conceptualized as an average tumor volume between \( t_a \) and \( t_b \), was therefore calculated as

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2 To whom requests for reprints should be addressed.

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Parameter A is functionally related to the ratio of growth rates in the perturbed (treated) versus unperturbed (control) situation, as previously described (18). Values for A for each tumor line and each therapeutic situation were transformed logarithmically to stabilize variances, compared by analysis of variance, and then compared pairwise by Student's t tests, one-tailed.

Patients. Twelve consecutive patients with malignant mesothelioma were treated between the end of 1981 and the beginning of 1983 with the best regimen found in the nude mouse system. Pretreatment evaluation procedures, performance status categories, and criteria of response to chemotherapy using clinically palpable tumors, roentgenograms, and/or computed tomography, have been previously described (8). All patients had advanced disease at the initiation of chemotherapy (Stages III and IV from a TNM (tumors-nodes-metastases) classification (4) for patients with pleural mesothelioma). Ten patients had a history of prior asbestos exposure. Cell type was epithelial, except for Patient 1, who had a biphasic or mixed type. All patients gave informed consent. All patients had normal hematological and renal functions. The initial 5 patients had all been treated previously with doxorubicin-containing regimens and were in progression. In Patients 1 to 4, these regimens included doxorubicin, 25 mg/m²/day i.v. on Days 1 to 3, and 5-azacytidine, 120 mg/m²/day by continuous i.v. infusion on Days 1 to 5, cycles being repeated every 4 weeks (8). After 7 cycles, doxorubicin was discontinued and replaced by cyclophosphamide, 200 mg/m²/day i.v. on Days 1 to 3. The total number of such cycles was 11 for Patient 1, 22 for Patient 2, 21 for Patient 3, and 23 for Patient 4. Patient 5 was previously treated with a combination of doxorubicin (45 mg/m²/day i.v.) and cyclophosphamide (450 mg/m²/day i.v.) once every 4 weeks for 10 cycles.

RESULTS

Nude Mouse System. Initial results of single agent chemotherapy in nude mice bearing human malignant mesothelioma xenografts revealed that 5-azacytidine, doxorubicin, 5-fluorouracil, methotrexate, vincristine, and vindeosine were inactive for both lines ES and BG. Active single agents for ES (by decreasing order of activity) were mitomycin C, dacarbazine, 1,3-bis(2-chloroethyl)-1-nitrosourea, bleomycin, and cyclophosphamide. Only cisplatin and bleomycin (by decreasing order of activity) were active in line BG. A combination of mitomycin C and cisplatin was the most active regimen for both lines ES and BG in nude mice. No difference was seen between mice bearing one or 4 xenografts each.

Quantification of antineoplastic effect in a representative experiment shown in Chart 1 and Table 2 (BG, 29th generation; ES, 31st generation) revealed that, for tumor line ES, there was no significant difference between control tumors and those treated with cisplatin. Mitomycin C caused tumors smaller than those of controls, but this did not achieve statistical significance in this particular experiment, where the number of tumors was small (p = 0.1). The combination of the ineffective drug cisplatin with mitomycin C, however, produced a significant difference between tumors so treated and controls (p < 0.005). In addition, tumors treated with the combination were significantly different from those treated with mitomycin C alone (p < 0.01). Tumor

Table 1

<table>
<thead>
<tr>
<th>Line BG</th>
<th>Line ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agents</td>
<td>% T/C</td>
</tr>
<tr>
<td>Mitomycin C, 1.5 mg/kg/wk i.p. for 3 wk</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>Cisplatin, 4 mg/kg/wk i.p. for 3 wk</td>
<td>17 ± 6</td>
</tr>
<tr>
<td>Mitomycin C, 1.5 mg/kg/wk i.p. for 3 wk, and cisplatin, 4 mg/kg/wk i.p. for 3 wk</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

* Generations 29 and 29, 8 to 15 tumors/group.
* Generations 31 and 32, 12 to 13 tumors/group.
* T/C, ratio of treated tumors to control tumors; ILS, increase in mean life span of treated animals compared to control animals.
* Mean ± S.E.
* p < 0.01 by Student's t test.

Chart 1. Growth curves of human malignant mesothelioma xenografts in nude mice. Results of one experiment in lines BG and ES. Treatments were all given i.p. once a week for 3 weeks (arrows), starting on Day 13 for BG and on Day 20 for ES. C, controls treated with 0.9% NaCl solution; M, mitomycin C, 1.5-mg/kg dose; P, cisplatin, 4-mg/kg dose; M + P, combination of mitomycin C and cisplatin at the same doses as above. Each treatment group includes 2 mice with 4 s.c. xenografts each. Bars, S.E. of the mean tumor volume which were generated using Student's t test distribution.
line BG displayed an opposite pattern of sensitivity. While cisplatin was ineffective against line ES, mitomycin C was ineffective against line BG. However, tumors of line BG treated with cisplatin were significantly smaller than those of controls \((p < 0.05)\). The combination of mitomycin C and cisplatin in line BG caused a further effect, so that tumors so treated were significantly different from those treated with cisplatin alone \((p < 0.01)\), and of course, from controls \((p < 0.0005)\). Hence, for each tumor line, the ineffective drug (cisplatin for ES and mitomycin C for BG) significantly augmented the antineoplastic effect of the other effective drug of this combination (Table 2).

Clinical Investigations. Following these experiments in nude mice, it was decided to evaluate the most active regimen, a combination of mitomycin C and cisplatin, in patients with malignant mesothelioma. Treatment included mitomycin C, 10 mg/sq m i.v., and cisplatin, 50 mg/sq m i.v., after adequate i.v. hydration. Antiemetic agents usually included a combination of metoclopramide (1 mg/kg i.v. every 4 hr for 4 doses, then as needed, and started 4 hr before cisplatin) and lorazepam (0.04 mg/kg i.v. once, 1 hr before cisplatin). Cycles were repeated every 4 weeks in the absence of residual toxicity. Dose adjustments for mitomycin C in case of residual hematological toxicity were as follows: 100% if peripheral blood leukocytes were 4,000 or more per \(\mu\)l and/or platelets were over 100,000/\(\mu\)l; 50% if leukocytes were between 3,000 and 3,999, and/or platelets were between 80,000 and 99,000; 25% if leukocytes were between 2,000 and 2,999, and/or platelets were between 70,000 and 79,000; and 0% if leukocytes were below 2,000, and/or platelets were below 70,000. Initial patients had failed prior chemotherapy with doxorubicin-containing combinations (Table 3). After the response seen in Patient 1 (Fig. 2), it was decided to use this regimen for previously untreated patients as well. Overall results showed objective response in 4 of 12 patients (one complete and 3 partial responses), occurring in 1 of 5 previously treated and 3 of 7 previously untreated patients (Table 3). Responses were usually slow to occur, requiring an average of 3 cycles. Patient 10 relapsed after 7 cycles, and subsequently responded to a combination of doxorubicin and 5-azacytidine.

Toxicity was moderate. Thrombocytopenia (platelets <100,000/\(\mu\)l) occurred in 7 patients, with a median nadir at 45,000 (range, 24,000 to 73,000). Four of these patients had previously been treated with chemotherapy. Leukopenia (leukocytes <3,000/\(\mu\)l) occurred in 4 patients (range, 2,600 to 2,900), including 2 previously treated patients. Hematological toxicity was usually reversible within 2 weeks, but dose adjustments were made if necessary. Nausea and vomiting were expected with cisplatin but were tolerable with the use of antiemetics. Serum blood urea nitrogen and creatinine, urinalysis, and creatinine clearance were obtained before each cycle. Renal toxicity occurred in only one patient with a serum blood urea nitrogen at 37 mg/100 ml and serum creatinine at 2.1 mg/100 ml after 6 cycles of therapy. Patient 7 developed acute shortness of breath and bilateral pulmonary infiltrates after the fourth cycle of chemotherapy, while in complete remission. He responded quickly to prednisone, but mitomycin C, the most probable responsible agent (3, 20), was discontinued. He relapsed within 3 months, and subsequently failed to respond to doxorubicin and cisplatin.

**DISCUSSION**

Malignant mesothelioma is notorious for its poor prognosis. Although some anecdotal cases of prolonged survival without treatment have been reported, median survival usually does not reach 1 year from diagnosis (4, 5, 8). Few chemotherapeutic agents have been clinically evaluated (4, 5, 8). Among them doxorubicin, alone and in combination, has been the most widely used. Our own results with such therapy have been disappointing. A response rate of 21% was obtained in 14 patients with

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**Table 2**

<table>
<thead>
<tr>
<th>Xenografts</th>
<th>Controls</th>
<th>Mitomycin C</th>
<th>Cisplatin</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line ES</td>
<td>2480 ± 606</td>
<td>1510 ± 278</td>
<td>1890 ± 443</td>
<td>706 ± 121</td>
</tr>
<tr>
<td>Line BG</td>
<td>934 ± 176</td>
<td>920 ± 179</td>
<td>475 ± 94</td>
<td>183 ± 29</td>
</tr>
</tbody>
</table>

\(\text{Mean} \pm \text{S.E.}\)

---

**Table 3**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Site</th>
<th>Performance status (d)</th>
<th>Prior chemotherapy (d)</th>
<th>No. cycles of mitomycin C and cisplatin</th>
<th>Response (d)</th>
<th>Duration of response (mos)</th>
<th>Survival from first mitomycin C and cisplatin (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>63</td>
<td>Pleura, left</td>
<td>3</td>
<td>Yes</td>
<td>10</td>
<td>Partial (d)</td>
<td>9</td>
<td>12</td>
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<tr>
<td>2</td>
<td>M</td>
<td>59</td>
<td>Pleura, right</td>
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<td>Yes</td>
<td>11</td>
<td>Stable (\geq 15)</td>
<td>&gt;15</td>
<td>&gt;15</td>
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<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>Peritoneum</td>
<td>1</td>
<td>Yes</td>
<td>7</td>
<td>Stable (\geq 8)</td>
<td>&gt;8</td>
<td>&gt;8</td>
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<tr>
<td>4</td>
<td>M</td>
<td>63</td>
<td>Pleura, right</td>
<td>3</td>
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<td>2</td>
<td>Progression (\geq 4)</td>
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<tr>
<td>5</td>
<td>M</td>
<td>58</td>
<td>Peritoneum and pleura, right</td>
<td>4</td>
<td>Yes</td>
<td>1</td>
<td>Progression (\geq 2)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>60</td>
<td>Pleura, left</td>
<td>1</td>
<td>No</td>
<td>10</td>
<td>Partial (d)</td>
<td>13</td>
<td>&gt;15</td>
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<tr>
<td>7</td>
<td>M</td>
<td>52</td>
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<td>1</td>
<td>No</td>
<td>4</td>
<td>Complete (\geq 6)</td>
<td>12</td>
<td>&lt;6</td>
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<tr>
<td>8</td>
<td>M</td>
<td>52</td>
<td>Pleura, left</td>
<td>3</td>
<td>No</td>
<td>4</td>
<td>Partial (\geq 4)</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>48</td>
<td>Pleura, left</td>
<td>1</td>
<td>No</td>
<td>4</td>
<td>Stable (\geq 3)</td>
<td>3</td>
<td>&gt;3</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>61</td>
<td>Pleura, left</td>
<td>0</td>
<td>No</td>
<td>7</td>
<td>Stable (\geq 6)</td>
<td>6</td>
<td>&gt;13</td>
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<tr>
<td>11</td>
<td>M</td>
<td>78</td>
<td>Pleura, right</td>
<td>0</td>
<td>No</td>
<td>8</td>
<td>Stable (\geq 7)</td>
<td>7</td>
<td>&gt;7</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>60</td>
<td>Pleura, right</td>
<td>2</td>
<td>No</td>
<td>4</td>
<td>Progression (\geq 6)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

\(\text{a} \): Classified according to the Cancer and Leukemia Group B scale: 0, ambulatory without symptoms; 1, ambulatory with symptoms; 2, bedridden less than 50% of daytime; 3, bedridden more than 50% of daytime; 4, totally bedridden.

\(\text{b} \): Doxorubicin, 5-azacytidine, and cyclophosphamide in Patients 1 to 4; doxorubicin and cyclophosphamide in Patient 5.

\(\text{c} \): Complete response, complete regression of all clinical and radiological evidence of disease; partial response, partial regression with more than 50% decrease in the sum of 2 perpendicular diameters of measurable lesions without appearance of a new lesion elsewhere; stable disease, less than 50% regression of measurable lesions, or stabilization, or regression of evaluable but not measurable lesions; progression, clinical and/or radiological progression with more than 25% increase in measurable lesions.

\(\text{d} \): Denotes presence of measurable lesion(s).
pleural malignant mesothelioma treated with high-dose doxorubicin (90 mg/sq m over 3 days) combined with radiotherapy (8). Another trial of doxorubicin (60 mg/sq m every 3 weeks) combined with radiotherapy yielded only one measurable response among 10 patients with pleural malignant mesothelioma (27). In a cooperative group trial, objective response to doxorubicin was seen in only 7 of 51 patients (14%) with malignant mesothelioma (16). A more recent trial comparing 2 dose schedules of doxorubicin alone (70 mg/sq m every 3 weeks, or 20 mg/sq m on Days 1 to 3 and 15 mg/sq m weekly) versus a combination of doxorubicin (60 mg/sq m on Day 1) and dacarbazine (250 mg/sq m on Days 1 to 3) every 3 weeks yielded a total of only 7 of 66 objective responses (11%), with no differences among the 3 treatment programs (15). The evaluation of other regimens of chemotherapy is therefore highly desirable. The small number of patients with malignant mesothelioma, however, precludes a systematic evaluation of these therapies. It is unlikely that empirical clinical trials will lead to the discovery of active regimens for such a refractory disease.

Development of a predictive system as an experimental model for malignant mesothelioma may circumvent this problem. A hamster mesothelioma obtained by i.p. injection of asbestos fibers has been described (14), but such tumors are not of human origin. In vitro clonogenic assays have met with limited success in mesothelioma (31). The cloning efficiency of human malignant mesothelioma in such a system seems to be low (28). Recently, a number of human tumors have been successfully transplanted into immune-deficient mice (13, 21, 22, 29), including malignant mesothelioma (1, 6). Such xenografts usually retain the original human morphological and biological features, and results of chemotherapy in other tumor types have correlated rather well with clinical experience (13, 21, 22, 29), and even with results obtained in the original donors (26). The advantages of the nude mouse model for a rare tumor such as mesothelioma, for which fresh specimens are not routinely or repeatedly available, are obvious. The xenografts can be serially transplanted for prolonged periods of time, they constitute a permanent source of fresh tissue, and they allow the screening of many agents alone and in combination. Doxorubicin did not show activity against lines BG and ES in our nude mouse model. After exploratory thoracotomy and transplantation of the tumor into nude mice, Patient B. G. was treated with a combination of doxorubicin and 5-azacytidine without any response. Patient E. S. died shortly after surgery without receiving chemotherapy.

Our results with mitomycin C and cisplatin in 12 patients with malignant mesothelioma seem to confirm the usefulness of the nude mouse model for this disease. Some clinical data are available for cisplatin alone in malignant mesothelioma, including one complete response from 9 patients with pleural mesothelioma in one trial (11), and one complete response out of 6 patients in another (24). No meaningful information is available regarding the activity of mitomycin C as a single agent. Our decision to evaluate clinically the combination of mitomycin C and cisplatin was entirely based on the nude mouse model, where this combination was the most active for both lines. The limited number of patients with malignant mesothelioma did not allow exploration of multiple dose schedules, nor random comparison with either drug given alone in an attempt to fully verify the experimental results obtained in the nude mouse model.

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Fig. 1. Nude mouse with 4 human malignant mesothelioma xenografts, Line ES, first generation, Day 68 after tumor implantation. The s.c. tumors are easily measurable and grew progressively faster after serial transplantation in nude mice.

Fig. 2. Chest roentgenogram of patient 1, left pleural mesothelioma, biphasic type. A, September 1981, immediately before mitomycin C and cisplatin, during progression after 11 cycles of doxorubicin and 5-azacytidine; B, January 1982, after 5 cycles of mitomycin C and cisplatin.
Experimental and Clinical Activity of Mitomycin C and cis-
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