Timing of Chemotherapy and Surgery in a Murine Osteosarcoma Model

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ABSTRACT

The sequential use of chemotherapy and surgery in the treatment of osteosarcoma developed in an empirical fashion without the benefit of investigations in animal models. The MGH-OGS murine osteosarcoma is a transplantable tumor that resembles the human disease with respect to histology, local invasiveness, metastatic characteristics, tumor ploidy, and its response to chemotherapy. We have used this tumor model to investigate the efficacy of preoperative, perioperative, and postoperative chemotherapy on the development of pulmonary metastases in three different experimental protocols. In each experimental design, perioperative chemotherapy demonstrated a significant advantage in preventing systemic relapse.

INTRODUCTION

The prognosis for patients with high grade osteosarcoma has improved dramatically in the past 20 years (1). Several centers now report 2-year disease-free survival in more than 65% of their patients, in contrast to reviews conducted 3 decades ago which demonstrated that 80% of patients died of their disease within 2 years of presentation (1–7). Although there was initial controversy related to the role played by chemotherapy in improving outcome in osteosarcoma (8), most authorities now accept that chemotherapy is efficacious and a prospective randomized trial has shown a clear survival advantage in patients receiving postoperative adjuvant chemotherapy compared with patients treated with surgery alone (4).

Several groups (3, 5–7) have published uncontrolled studies indicating that preoperative “neoadjuvant” chemotherapy may be more effective than postoperative treatment in improving systemic relapse in osteosarcoma. This apparent improvement in results by using chemotherapy prior to surgery suggests that the temporal relationship between surgery and chemotherapy may be an important variable in determining outcome in osteosarcoma. Ragaz et al. (9), in discussing neoadjuvant chemotherapy in breast cancer, declared that it is unlikely that new cytotoxic agents with dramatically improved tumoricidal characteristics will appear in the near future. It is therefore fundamental to determine the optimal timing for the administration of currently available chemotherapy medications (9).

It should be recognized that current chemotherapy protocols for osteosarcoma have developed in empirical fashion with little or no experimentation in animal models prior to clinical trials. This paper describes a transplantable murine osteosarcoma that appears to be an excellent model for the human disease in both its biological behavior and its response to chemotherapy. We also report investigations of the timing of chemotherapy and surgery that suggest that perioperative (as opposed to preoperative or postoperative) administration of cytotoxic drug may enhance the effect of chemotherapy.

MATERIALS AND METHODS

The MGH-OGS transplantable murine osteosarcoma originated in a previously irradiated C3H-Sed mouse (10). The original first generation tumor was stored in liquid nitrogen. In these experiments, the first generation tumor was rapidly thawed and implanted in irradiated mice. The resultant second generation lesions were transplanted to nonirradiated animals which served as a tumor source for the experiments reported below. Fourth and fifth generation tumor was consistently used in these studies.

All animals were housed in the defined flora colonies of the Edwin L. Steele Radiation Biology Laboratory of the Massachusetts General Hospital or the Sarcoma Biology Laboratory of St. Michael's Hospital. Tumors were transplanted by mincing third or fourth generation source lesions in Ham’s F-12 medium (GIBCO, Grand Island, NY), and implanting 1-mm fragments in the lateral gastrocnemius muscle of 8-week-old C3H-Sed mice. After implantation, tumor volume was estimated by measuring 3 orthogonal diameters of the lesion and multiplying this product by π/6.

All surgical procedures were undertaken with general i.p. sodium pentobarbitol anesthesia. Amputations were performed by tightly applying a Kocher forceps proximal to the lesion and amputating the limb with Stevens’s scissors. Hemostasis was achieved by using cautery and the skin edges were approximated with surgical staples. No manipulation of the tumor was attempted prior to applying the forceps which completely occluded arterial inflow and venous outflow to and from the tumor.

One experimental group of animals underwent marginal tumor resection. The technique for this form of limb salvage surgery is described below.

Characterization of MGH-OGS Growth, Histology, Ploidy, and Metastatic Rate. The mean growth curve of the MGH-OGS was determined by three weekly measurements of tumors in 175 animals used as controls in various experiments and then plotting these measurements against time from implantation by using semilogarithmic paper. One hundred and twenty of these tumors were harvested after amputation or sacrifice and were fixed in formalin, decalciﬁed in formic acid, sectioned, and stained with hematoxylin and eosin. The histological characteristics of the tumor were reviewed by an experienced musculoskeletal pathologist (A. E. R.).

To study the relationship between primary tumor size at the time of treatment and the eventual incidence of metastases, 120 animals underwent amputation at 4, 6, 8, 12, or 16 mm mean diameter. These animals were followed up to 9 months when all survivors were sacrificed and autopsied with attention to the presence of pulmonary and skeletal metastases or locally recurrent disease. All animals that died prior to 9 months also underwent autopsy.

The primary tumors removed at various sizes were used to investigate the DNA content of MGH-OGS cells by flow cytometry. Following amputation, the tumors were dissected free of the surrounding normal tissues, minced with scissors in Ham’s F-12 medium, and digested in 0.09% collagenase (Worthington). The cells were collected by centrifugation, resuspended at 10⁶ cells/ml, and their nuclei were stained with propidium iodide in the presence of Triton X-100 and ribonuclease (11). The cellular DNA content was evaluated in a flow cytometer, with the resultant DNA histograms being evaluated in primary tumors of various sizes and in metastatic lesions removed from the lungs.

Adriamycin Effect on the Primary Tumor. Adriamycin was administered by i.p. injection. One hundred animals were weighed daily from

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day 5 to day 30 after injection to evaluate the drug effect on body mass. Four experiments, all using a single drug injection, were performed to evaluate the effect of Adriamycin on primary tumor growth. In the first experiment, 0.012 mg/g Adriamycin was administered to small tumors (4–8 mm mean diameter) or large tumors (8–12 mm), and growth delay was calculated from mean growth curves of the treated and control tumors. Adriamycin was then given at 10-mm mean tumor size in varying doses (0.004, 0.008, or 0.012 mg/g Adriamycin) to evaluate a dose response effect. The histological effect of Adriamycin was evaluated by harvesting tumors from 20 animals 2 weeks after they had been treated with 0.012 mg/g at 6-mm mean diameter. The specimens were treated as described above and four sections were obtained from standardized regions of the specimen. These sections were evaluated by our pathologist (without knowledge of prior treatment) and necrosis was quantitated as described by Rosen et al. (6). Twenty control tumors were processed and evaluated in similar fashion.

Finally, in order to evaluate the possible effect of pentobarbital anesthetic on the efficacy on Adriamycin, 60 animals were randomized to receive no drug, Adriamycin alone, or Adriamycin and pentobarbital (20 animals/group) at 8-mm mean tumor size. The Adriamycin and pentobarbital were given sequentially by separate i.p. injections. The mean growth curves and growth delay were calculated for the treatment groups.

Adriamycin Effect on Systemic Relapse: Evaluation of the Timing of Chemotherapy and Surgery. Three experiments evaluated the effect of Adriamycin (0.01 mg/g) on systemic relapse. The last of these three investigations also evaluated the drug effect on local relapse following marginal resection.

All of these experiments utilized 4 treatment groups: Group A, amputation (or marginal resection) without chemotherapy; Group B, chemotherapy followed 2 weeks later by surgery (preoperative treatment); Group C, surgery followed 2 weeks later by chemotherapy (postoperative treatment); and Group D, simultaneous chemotherapy and surgery (perioperative treatment).

The 2-week delay between chemotherapy and surgery was chosen since previous experiments had demonstrated that the growth plateau in the primary tumor following the administration of Adriamycin lasted at least 3 weeks.

In order to maximize the risk of metastases, animals in the first experiment were entered into the protocol at a mean tumor size of 16 mm. As described above, amputations were performed by cross-clamping the extremity proximal to the tumor prior to any manipulation of the lesion. Venous outflow was thereby blocked prior to resection. Animals were entered into each treatment group sequentially to avoid bias. These animals were followed for 8 months, when all remaining survivors were sacrificed. Cages were checked daily and immediate autopsies (gross evaluation of the lungs, abdomen, spine, and extremities) were performed on all animals that died or were sacrificed at the end of the experiment.

This protocol was repeated by using an identical protocol (treatment starting at 16-mm tumor size) and a different end point for evaluation of treatment effect. In this second experiment, all animals were sacrificed at 63 days after entry into protocol and their lungs were weighed and fixed in Bouin's solution. After all lungs had been harvested and fixed, surface pulmonary metastases were measured with calipers and the percentage of lung surface area involved by tumor was calculated. In calculating surface area, the metastases were assumed to be circles and the lung surfaces triangles. Areas were then calculated geometrically after measurements had been taken. After visual inspection of the lungs, the specimens were sectioned and stained with hematoxylin and eosin to verify the presence of metastases.

In the final experiment in this series, animals were entered into the same 4 treatment protocols at 6-mm mean tumor size. However, instead of amputation, marginal resection was used to gain local control. All resections were performed by the same orthopedic oncology surgeon, unaware of treatment group. Each resection was carried out at the pseudocapsule of the tumor. In this fashion, all gross tumor was excised but microscopic disease remained in the wound. All wounds were checked for local recurrence 3 times weekly and the extremity was amputated immediately if local relapse was detected. The animals were then followed for 6 months prior to sacrifice. Autopsies were performed on all animals that died prior to 6 months and on the survivors. These autopsies included gross and microscopic analysis of the resected primary tumor site and estimation of the lung surface area involved by tumor as described earlier.

RESULTS

Tumor Growth, Histology, Ploidy, and Metastases. To date, transplantation has resulted in successful tumor growth in 1269 of 1350 animals (94%). Since the transplant is inserted through a small skin incision, the inoculum occasionally is dislodged by the animal and this probably accounts for the 6% failure rate in transplantation. As seen in Fig. 1, the 95% confidence intervals for the MGH-OGS growth curve are fairly wide, probably as a result of the wide variation in tumor cell number within the transplanted 1-mm fragment.

The MGH-OGS is a highly cellular neoplasm with irregular trabeculae of mineralized tumor osteoid predominating in the central portion of the lesion (Fig. 2). At the periphery of the lesion, neoplastic cells can be seen to infiltrate skeletal muscle and, in most implants, there is a peripheral 200- to 300-μm rim of cells that have not yet produced bone.

Cells isolated from the MGH-OGS tumor were essentially diploid on flow cytometric evaluation with a growth fraction (i.e., S-phase plus G2-M cells) that was typically less than 5%. A small aneuploid cell population at DNA index 1.4 was visualized in 90 of 100 primary tumors analyzed for cellular DNA. This small aneuploid population however, never exceeded 8% of the total cell number. There was no significant difference in cell cycle distribution in histograms obtained from 4-, 6-, 8-, 12-, or 16-mm tumors. Metastatic tumors were generally diploid and in 25 of 34 metastatic lesions analyzed, there was no evidence of an aneuploid cell population. The aneuploid cells noted in 9 metastatic tumors had a DNA index identical to the primary lesion (Fig. 3).

The incidence of metastases was directly related to the size of the primary tumor at amputation (Fig. 4). Of mice with tumors amputated at 16 mm, 96% had developed metastases by 6 months. Animals that survived to 6 months showed no evidence of pulmonary disease when autopsied at 9 months after treatment of the primary lesion. All tumor deaths in these experiments were associated with intrathoracic hemorrhage from pulmonary lesions. There were no cases of local recurrence after amputation of the primary lesion. In 368 autopsies per-
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formed to date there have been 12 cases of bony metastasis (all to the lumbar spine).

Adriamycin Effect on Primary Tumor. Adriamycin (0.012 mg/g) caused an average weight loss of 3.2 ± 1.4% (SD) that was maximal at 17 days after i.p. injection (range, 14–23 days). Mortality from injection (defined as occurring within 48 h of the injection) was low (4 deaths in 546 injections). Mortality in unoperated animals within 4 weeks of injection was 3.3% (4 of 120 animals). Adriamycin resulted in a mean growth delay (determined at 16-mm mean tumor diameter) of 30.9 ± 8.46 days in 4- to 8-mm tumors and 20.3 ± 6.74 days in 8- to 12-mm tumors (Fig. 5). Tumor size in the treatment groups was significantly less than the control group from day 7 after treatment onward in both tumor sizes treated (P < 0.01, Student t test).

A significant dose response effect was found in administering 0.004, 0.008, or 0.012 mg/g Adriamycin (Fig. 6). Tumor size measured at 14 days after injection demonstrated a significant inverse correlation with the dose of drug injected (R² = 0.91, P < 0.01).

Several effects of Adriamycin on tumor histology were evident 2 weeks after treatment of 8-mm tumors with 0.012 mg/g. Large areas of necrosis were identified with the osteoid matrix devoid of cells in the treated tumors (Fig. 7), whereas no necrosis was found in untreated control lesions. Both the peripheral rim of undifferentiated cells and the degree of skeletal muscle invasion were markedly reduced in the treated tumors and there was an increase in the calcified osteoid matrix found in the central portion of the tumor.

The simultaneous administration of Adriamycin and pentobarbital did not alter the drug efficacy in delaying primary

![Fig. 2. The central portion of the tumor was heavily calcified, with irregular trabeculae of tumor osteoid separating nests of tumor cells. H & E, × 200.](image)

![Fig. 3. DNA histograms from 90% of primary tumors demonstrated a small aneuploid peak with a predominance of diploid cells. Metastatic lung tumors generally failed to show an aneuploid peak.](image)

![Fig. 4. Mean diameter of the lesion (mm) at amputation of primary tumor determined risk of metastases.](image)

![Fig. 5. Adriamycin treatment (0.012 mg/g) of the primary tumor resulted in growth delay in both small (4- to 8-mm) or large (8- to 12-mm) tumors. Delay in reaching 16-mm mean diameter was 30.9 days in smaller tumors and 20.3 days in the larger lesions. Vertical bars represent 95% confidence limits.](image)

![Fig. 6. Growth delay in the primary tumor (10 mm) was dependent on the dosage of Adriamycin (Adria) administered.](image)
tumor growth. There was no significant difference in tumor volume found at any time point after drug administration. The mean growth delay for animals treated with Adriamycin alone was 27.2 ± 6.51 days compared with 25.7 ± 5.95 days in mice receiving Adriamycin and pentobarbital (P = 0.67, Student t test).

Adriamycin failed to arrest tumor growth in any animal completely. Each lesion resumed growth after a delay that varied according to the drug dose administered, and the size of the lesion at the time of treatment.

Effect of Preoperative, Postoperative, and Perioperative Adriamycin on Systemic Relapse. In the investigation of the effect of Adriamycin on animal survival (protocol entry at 16-mm mean tumor diameter), chemotherapy significantly prolonged survival with the magnitude of drug effect dependent on the timing of chemotherapy administration (Table 1). Mean survival in animals treated with surgery alone was 55.61 ± 25.1 days, compared to 74.25 ± 25.60 days in the preoperative chemotherapy group, 71.32 ± 32.6 days in the postoperative chemotherapy group, and 103.08 ± 27.74 days in animals receiving perioperative treatment. Analysis of variance demonstrated significant intergroup differences in survival (P < 0.01), the Duncan multiple range test showed perioperative treatment to be better than any of the other three treatment regimens, and both preoperative and postoperative chemotherapy to be superior to surgery alone.

The improved efficacy of perioperative chemotherapy was confirmed in the second experiment when the animals were sacrificed 9 weeks after entering protocol and their lungs were weighed and examined. Five animals in this experiment died prematurely (four in the group treated with surgery alone died of extensive pulmonary disease and one animal treated with preoperative Adriamycin died without metastases 13 days after receiving the drug). Animals treated with surgery alone had mean lung wet weights almost twice that of the groups receiving Adriamycin (P < 0.01, analysis of variance, and Duncan multiple range test). The proportions of animals with grossly visible metastases were 27 of 27 (100%) for surgery alone, 17 of 26 (65%) for preoperative treatment, 21 of 33 (64%) for postoperative treatment, and 5 of 32 (16%) for perioperative treatment. χ² analysis demonstrated that perioperative treatment was superior to any of the other groups (P < 0.01) and that preoperative and postoperative treatment were superior to surgery alone (P < 0.01) (Table 2).

The percentages of lung surface area involved by tumor for each group can be found in Fig. 8.

Histological analysis of the lungs confirmed that the lesions interpreted as metastases demonstrated histology identical to that of the primary tumor.

The final experiment in this series demonstrated the benefit of perioperative chemotherapy with marginal resections in smaller tumors (6 mm). Local recurrences occurred in 17 of 22 animals treated with surgery alone, compared to 8 of 25 local relapses in the postoperative chemotherapy group, 1 of 22 in animals treated with preoperative chemotherapy, and 1 of 26 in the perioperative group. χ² testing again demonstrated that the latter two groups had significantly fewer local failures than animals treated with postoperative chemotherapy (P < 0.05) or no chemotherapy (P < 0.01). Systemic relapses occurred in 19 of 22 (surgery alone), 6 of 22 (preoperative chemotherapy), 11 of 25 (postoperative chemotherapy), and 1 of 25 (perioperative chemotherapy). χ² testing again demonstrated a significant advantage for perioperative chemotherapy. If systemic relapse was evaluated in animals without evident local recurrence, the metastatic incidence was 5 of 5 (surgery alone), 6 of 21 (preoperative chemotherapy), 7 of 17 (postoperative chemotherapy), and 1 of 25 (perioperative chemotherapy). χ² testing again confirmed the benefit of perioperative treatment compared to the other groups (P < 0.05) (Table 3).

In this study, all animals treated with chemotherapy who relapsed systemically had lung lesions measuring less than 10% of the total lung surface area. Histological examination was performed in each case to verify the presence of tumor in these small lung lesions.

Table 1 Effect of Adriamycin (0.01 mg/g) on systemic relapse

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No.</th>
<th>Mean survival ± SD (days from onset of treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Surgery alone</td>
<td>29</td>
<td>56 ± 25.1*</td>
</tr>
<tr>
<td>B</td>
<td>Preoperative chemotherapy</td>
<td>29</td>
<td>74 ± 25.6</td>
</tr>
<tr>
<td>C</td>
<td>Postoperative chemotherapy</td>
<td>24</td>
<td>71 ± 32.6</td>
</tr>
<tr>
<td>D</td>
<td>Perioperative chemotherapy</td>
<td>26</td>
<td>103 ± 27.7</td>
</tr>
</tbody>
</table>

* P < 0.01, significant intergroup differences (analysis of variance). Duncan multiple range test: D differs significantly from A, B, C; B differs significantly from A; C differs significantly from A.
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Table 2 Effect of Adriamycin (0.01 mg/g) on systemic relapse

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No.</th>
<th>Mean lung wet wt ± SD (mg)</th>
<th>Metastases</th>
<th>Mean % of lung surface occupied by metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Surgery alone</td>
<td>27</td>
<td>3013 ± 812</td>
<td>27 (100)</td>
<td>26.9*</td>
</tr>
<tr>
<td>B</td>
<td>Preoperative chemotherapy</td>
<td>26</td>
<td>1663 ± 554</td>
<td>17 (65)</td>
<td>7.3</td>
</tr>
<tr>
<td>C</td>
<td>Postoperative chemotherapy</td>
<td>33</td>
<td>1598 ± 433</td>
<td>21 (64)</td>
<td>8.2</td>
</tr>
<tr>
<td>D</td>
<td>Perioperative chemotherapy</td>
<td>32</td>
<td>1542 ± 384</td>
<td>21 (64)</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Analysis of variance: significant intergroup differences in lung weights (P < 0.01) and percentage of lung surface occupied by metastases (P < 0.01). Duncan multiple range test (wet lung weight): A significantly differs from B, C, D. X²: incidence of metastases: D differs from A, B, C (P < 0.01); A differs from B, C (P < 0.01). Duncan multiple range test (percentage of lung surface with metastases: D differs from A, B, C; A differs from B, C.

Table 3 Effect of Adriamycin (0.01 mg/g) on systemic relapse

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No.</th>
<th>Local relapse</th>
<th>Systemic relapse</th>
<th>Systemic relapse without local failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Surgery alone</td>
<td>22</td>
<td>17/22</td>
<td>19/22</td>
<td>5/5</td>
</tr>
<tr>
<td>B</td>
<td>Preoperative chemotherapy</td>
<td>22</td>
<td>1/22</td>
<td>6/22</td>
<td>6/21</td>
</tr>
<tr>
<td>C</td>
<td>Postoperative chemotherapy</td>
<td>25</td>
<td>8/25</td>
<td>11/25</td>
<td>7/17</td>
</tr>
<tr>
<td>D</td>
<td>Perioperative chemotherapy</td>
<td>26</td>
<td>1/26</td>
<td>1/25</td>
<td>1/25</td>
</tr>
</tbody>
</table>

X² analysis: D differs from A, C in 3 categories (P < 0.01); A differs from B, C in 3 categories (P < 0.01); D differs from B in systemic relapse (P < 0.05) in systemic relapse without local failure (P < 0.05).

Histological evaluation of the primary tumor site at autopsy demonstrated the presence of microscopic nodules of tumor in the 5 animals treated with surgery alone who demonstrated no clinical evidence of local recurrence. No microscopic tumor at the local site was found in any of the animals treated with chemotherapy who appeared free of local disease on clinical examination.

In this final experiment, 3 animals treated with surgery alone died in the immediate postoperative period, as did 3 animals in the preoperatively treated group. These animals were discarded from further analysis.

DISCUSSION

The experiments described above demonstrate that the MGH-OGS is a useful model for OSA. This tumor resembles human OSA in that it produces abundant neoblastic bone, invades skeletal muscle, and metastasizes preferentially to the lungs. Evaluation of cellular DNA content showed a minimal population of aneuploid cells, and studies in our laboratory have shown that 39% of human OSA fail to demonstrate a prominent aneuploid peak on flow cytometric ploidic analysis (12, 13). Similar to human OSA, the risk of developing metastasis was correlated with tumor size at the time of treatment.

The MGH-OGS is appropriate for studying chemotherapy effects in OSA since prolonged cessation of tumor growth can be achieved by a single dose of Adriamycin. Tumors treated by one dose of Adriamycin demonstrated extensive tumor necrosis, tumor calcification, and decreased peripheral muscular invasion. These changes have also been reported following successful preoperative chemotherapy in humans (3, 6, 7, 14, 15). The effectiveness of Adriamycin in controlling local tumor growth is dose dependent and related to tumor size at the time of treatment.

The investigation of the timing of chemotherapy and surgery was prompted by uncontrolled clinical studies that suggest that preoperative, neoadjuvant chemotherapy may effect systemic relapse rates to a greater extent than postoperative adjuvant treatment. The group receiving perioperative treatment was added as a further control arm currently not used in clinical practice. The substantially greater efficacy of perioperative treatment was confirmed in 3 separate experiments utilizing more than 300 animals, different end points (i.e., survival or metastatic incidence prior to death), different sizes of primary lesions, and different surgical methods. The apparent enhancement of drug effect when administered at the time of surgery is intriguing and warrants further discussion.

One possible explanation for the results reported here is that the pentobarbital anesthetic might retard Adriamycin clearance or alter the kinetics of Adriamycin uptake by MGH-OGS tumor cells. This appears unlikely, however, since simultaneous administration of Adriamycin and pentobarbital did not alter the growth delay in the primary tumor compared to Adriamycin given alone. Perioperative administration of Adriamycin did not increase perioperative mortality; indeed, the only animals to die following marginal resection (a more difficult and time consuming operation than amputation) were in the no chemotherapy and perioperative chemotherapy groups.

It could be suggested that perioperative chemotherapy would eliminate tumor cells shed into the venous circulation by manipulation of the lesion at surgery. This potentially protective effect of perioperative chemotherapy is not important in this model, however, since in the first two experiments described above, venous outflow from the tumor was interrupted prior to amputation by placing a forceps across the extremity proximal to the lesion.

An interesting explanation of the efficacy of perioperative chemotherapy is provided by work done by Fisher and other investigators on the effect of resection of a primary tumor on
residual metastatic disease. Fisher et al. (16, 17) have shown in a mouse mammary cancer, that resection of the primary lesion causes a marked increase in growth in secondary lesions and that this increased growth is mediated by the recruitment of resting cells into the cell cycle. This concept has been confirmed in other experimental tumor models (18–27). Recent studies of this phenomenon in the MGH-OGS have shown, in a dual tumor model, that enhanced growth and an increase in the tumor labeling index can be achieved in a secondary lesion by amputating the primary lesion (28).

Although the mechanism of this "recruitment" phenomenon is not understood, it is evident that if metastatic disease is stimulated by resection of the primary tumor, the perioperative period may represent a "window of opportunity" for the administration of cytotoxic drugs. Chemotherapy would be expected to be more effective if given when many cells were entering the cell cycle (16). Although this explanation of the effect of perioperative treatment may seem conjectural, it should be recognized that the effect of surgery on the kinetics of micrometastatic disease is considered important in clinical protocols for the adjuvant treatment of breast carcinoma, and that perioperative treatment of breast tumors is currently under clinical study (9).

The drug regimens in this study are not analogous to the current protocols used for the human osteosarcoma. Clinical protocols use multiple agents in repeated cycles in both the preoperative and postoperative periods. In this experiment, the use of a single course of chemotherapy given preoperatively may not have provided the lasting effect on micrometastatic disease that might have been apparent if multiple courses of preoperative treatment had been used. The intent of these experiments was to investigate biological principles rather than to evaluate any particular protocol. However, it does appear that the timing of operation relative to adjuvant therapy is important and that certain issues relevant to the treatment of osteosarcoma may be profitably examined in this murine model. Adjuvant chemotherapy protocols in osteosarcoma developed in an empirical fashion, and the use of preoperative, neoadjuvant treatment was initiated to permit time for the production of a custom prosthesis rather than from a consideration of tumor kinetics or the optimal timing for drug administration. The findings in this experimental study suggest that the sequencing of chemotherapy administration and surgery is very important in optimizing the effectiveness of combined treatment. Certainly, information gained from experimental models will be useful in understanding the effect of chemotherapy and surgery on tumor kinetics and may be helpful in planning new clinical trials.

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