A Role for Chemotherapy as an Adjunct to Surgery*

DANIEL M. SHAPIRO AND RUTH A. FUGMANN

WITH THE TECHNICAL ASSISTANCE OF P. HAYWORTH, L. MATHIES, A. PARISI, A. SCHNEIDMAN, AND A. SPUTO

(Department of Surgery, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

A number of chemotherapeutic agents which have effected "cures" of solid experimental tumors in the laboratory have had essentially no effect on a variety of solid tumors in the clinic (e.g., 6-mercaptopurine [1, 2, 5] and puromycin [6, 7]). This divergence in response has not yet been explicable and has led to grave concern over the possible existence of fundamental differences between spontaneous and transplanted tumors.

In considering this problem, it occurred to us that a possible explanation for this discrepancy in effect could lie simply in the methodology employed to determine such effect. The host (patient) studied in the clinic invariably has large, extensive tumor masses, whereas the host (animal) studied in the laboratory has small, discrete, recently implanted tumor masses.

To elucidate a possible relationship between tumor size and chemotherapeutic response, laboratory experiments were designed to assess the effect of such a chemotherapeutic agent (one capable of producing complete regression of a laboratory neoplasm) on tumors of progressively increasing size. The results of these experiments suggest that an inverse relationship between the number of solid tumor cells and the chemotherapeutic response exists.

MATERIALS AND METHODS

General.—C57BL mice, 2–4 months old and weighing 18–22 gm., were housed in plastic cages in an air-conditioned, constant-temperature room (74° F.). All animals had free access to Rockland pellets and water. Mammary adenocarcinoma 755, implanted subcutaneously into the axillary region by the usual trocar technic, was the neoplasm employed.

The chemotherapeutic agent chosen for these studies, 6-mercaptopurine (6-MP), was freshly prepared and injected intraperitoneally as a weakly alkaline solution. The standard course of therapy for all experiments was six injections of 6-MP at a dose of 40 mg/kg over a 2-week period (approximately every other day). All animals were sacrificed 2 weeks after cessation of 6-MP therapy. The tumors which had not undergone complete regression were removed, and wet weights were determined to the nearest milligram. All abnormal tissue, no matter how small in amount or equivocal as to its etiology or viability, was considered to be residual tumor and was removed and weighed.

Wherever indicated, a group of animals labeled "sacrificed controls" was killed on the day treatment was begun. The mean tumor weight of this group was obtained to provide an index of tumor size on the day therapy was begun.

Surgical.—All surgical procedures were performed on animals whose tumors were 15 days old. At this time the mice were anesthetized by the intraperitoneal injection of veterinary sodium nembutal at a dose of 85 mg/kg. The animals were then taped to a board, shaved in the general area of the tumor, and cleansed with 70 per cent alcohol. Under sterile conditions an incision was made in the skin, appropriate surgery was performed on the tumor, and the skin was closed with 11-mm. Michel Wound Clips. Depending on the specific experiment, either of two types of surgery was performed: (a) partial excision—the tumor was transected so as to deliberately leave some tumor behind; and (b) enucleation—an attempt was made to grossly strip out all tumor tissue. One week later any remaining wound clips were removed from the animals.

RESULTS

Experiments to determine effect of tumor size (or age) on chemotherapeutic response.—All mice were implanted with a single 755 tumor piece into the right axillary region and separated into five groups of twenty mice each by random selection. Two of these groups were "sacrificed controls," one terminated at 8 days (tumor weight = 6–7 mg.) and the other at 15 days (tumor weight = 118–251...
mg.) after tumor implantation. The three remaining groups received 6-MP therapy beginning 24 hours, 8 days, and 15 days after tumor implantation, respectively. Each group of treated animals was sacrificed 2 weeks after the standard course of 6-MP therapy; or 4 weeks, 5 weeks, and 6 weeks, respectively, after tumor implantation. The animals were examined by dissection to determine the percentage of tumors "cured."

The results of four experiments conducted in this manner were as follows: Therapy begun 24 hours after tumor implantation resulted in an average of 57 per cent "cures" (47–80 per cent); therapy begun 8 days after tumor implantation resulted in an average of 26 per cent "cures" (10–42 per cent); and therapy begun 15 days after tumor implantation resulted in no "cures" (Chart 1). At the end of the 2-week observation period, all tumors that were not "cured" had "escaped" and showed objective evidence of rapid growth.

The above results, showing a definite gradation in chemotherapeutic response, would appear to be primarily dependent on either of two factors: size of tumor or age of tumor at the onset of therapy. Therefore, further studies were initiated to explore the role of the age factor.

**Chart 1.** Decreasing chemotherapeutic (6-MP) "cure" rates with increasing tumor size (or age) (four experiments).

Experiments to assess the factors of age vs. size of tumor in chemotherapeutic response.—Experiments were performed to surgically reduce 15-day-old tumors (6-MP-incurable) so that they were smaller in size than 8-day-old tumors (6-MP-curable). Thus, the age (15 days) of the partially resected tumors would be retained, while the size would be reduced into the range of possible curability. The standard course of 6-MP therapy was administered beginning immediately following such partial surgical excision of the 15-day-old tumors. Chart 2 summarizes the results of three experiments conducted in this manner. While neither surgery alone nor 6-MP alone produced "cures" of 15-day-old tumors, the combination of the two resulted in a 57 per cent "cure" rate of the 15-day-old tumor cells.

These data ruled out age of tumor as being of primary importance in determining the observed enhancement of chemotherapeutic response following partial surgical excision. However, the observed "cure" rate might have been due to: (a) reduction of mass per se (i.e., from large tumor mass to a tiny tumor mass); (b) reduction of the total number of tumor cells per se; or (c) the influence of local surgical trauma, rendering the tumor cells more vulnerable to the chemotherapeutic agent.

**Chart 2.** Complete regression of a 15-day-old 755 tumor by surgical tumor reduction (partial surgical excision) plus chemotherapy (6-mercaptopurine).

Experiments to evaluate the influence of the following factors in chemotherapeutic response: (a) total number per se of tumor cells as opposed to mass per se (large vs. small) of tumor tissue, and (b) the influence of local surgical trauma.—Three experiments were performed with the use of a large tumor mass (15 days old) on one side of the animal to see if it would protect a small tumor mass (24 hours old) implanted into the opposite side of the same animal, from the known curative effect of 6-MP therapy on such small tumors. With the large tumor mass in position, an average "cure" rate of 14 per cent (5–22 per cent) of the tiny tumor mass was obtained with 6-MP therapy. When similar bilaterally transplanted mice had the large tumor mass surgically enucleated, 6-MP therapy resulted in an average "cure" rate of 72 per cent (40–100 per cent) of the tiny tumor mass (Table 1).

In addition, three similar experiments were performed with bilateral implants of 15-day-old tumors (large mass) and 5-day-old tumors (small mass), to determine whether such protection would occur when the small mass was well estab-
lished in regard to blood supply, etc. In this series of studies an average "cure" rate of the small tumor mass (5 days old) of 18 per cent (11-38 per cent) was obtained with the large tumor mass (15 days old) in position. On the other hand, an augmented average "cure" rate of the small tumor mass (5 days old) of 47 per cent (35-53 per cent) was obtained when the large tumor mass (15 days old) was surgically enucleated.

It would appear that a large tumor mass can protect a small tumor mass against 6-MP therapy in the same animal. If only mass were important in dictating chemotherapeutic response, then the small aggregate of tumor cells should have been equally susceptible to "cure" regardless of the presence in the host of a large tumor. Thus, the design of these experiments was such that the results ruled out individual tumor mass per se and local surgical trauma as factors strongly influencing chemotherapeutic response, clearly implicating the total number of tumor cells per se (regardless of aggregate size) as responsible for the observed titration of chemotherapeutic response.

**DISCUSSION**

The data indicate a definite relationship between total tumor mass and chemotherapeutic response, i.e., the less the number of tumor cells, the greater the chemotherapeutic effect. However, these data have thus far been obtained on a single solid tumor (755 breast carcinoma) with a single agent (6-mercaptopurine). Whether this relationship may be viewed as a general principle embracing many drugs and many tumors remains to be determined, although fragmentary evidence in support of such a possibility is recorded. Goldin et al. have reported that, as the number of cells in a leukemic inoculum was increased, it was necessary to increase the dosage of the drug in order to main-

**TABLE 1**

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Right side</td>
<td>Left side</td>
</tr>
<tr>
<td>Large tumor</td>
<td>Small tumor</td>
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* Bilateral tumor implants (755 breast carcinoma in C57BL mice). Right side implant allowed to grow to age of 14 days, then left side implant made. Standard course of 6-MP therapy initiated 1 day later. Group 2 differed from Group 1 only in that surgical enucleation of large tumor mass was performed on the day 6-MP therapy was initiated.

**REFERENCES**

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