Radiotherapy and Adjuvant Combination Chemotherapy (6-Aminonicotinamide and 6-Mercaptopurine)*

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As recently reviewed (1), combining chemical agents with radiotherapy to attain increased antitumor effects has often been attempted, and increased carcinostasis has been noted. More recently, an experimental study was reported in which augmented tumor damage, evidenced at the objective level of complete tumor regression ("cures"), was produced by combining radiotherapy and combination chemotherapy (3). At least one of the drugs employed in that combination (8-azaguanine) is known to be too toxic for human administration. The purpose of the present communication, therefore, is to present data demonstrating similar augmented "cure" rates by combining two clinically acceptable compounds, 6-mercaptopurine and 6-aminonicotinamide (2), with radiotherapy.

MATERIALS AND METHODS

Male C57BL mice, 2–4 months old and weighing 18–25 gm., were housed in plastic cages in an air-conditioned, constant-temperature room (74° F.) and received a diet of Rockland pellets and water, ad libitum. Mammary adenocarcinoma 755 was transplanted into the subcutaneous tissue of the left hind leg of each animal. These implants were allowed to grow for 17 days, at which time they were well established and of good size. Each animal was palpated to ascertain the presence of a tumor, and those not demonstrating a palpable tumor were eliminated. With reference to a table of random numbers, the animals were then separated into groups of approximately twenty animals each, and appropriate treatment was initiated.

Thus, beginning on the 17th day after tumor transplantation, all animals receiving chemotherapy, alone or in conjunction with radiotherapy, were treated once daily for 5 consecutive days. 6-Mercaptopurine (6-MP), as a slightly alkaline solution at a dose of 20 mg/kg daily, and 6-aminonicotinamide (6-AN), in saline, at a dose of 2 mg/kg daily, were administered intraperitoneally. All animals receiving radiotherapy, alone or in conjunction with chemotherapy, were treated over the same 5-day period and received a total tissue dose of 5,300 r, given in three equal doses on the 1st, 3d, and 5th treatment days. During the x-ray therapy the animals were encased in a lead ($\frac{1}{4}$-inch) box from which only the tumor-bearing hind leg protruded. This box was placed in a specially constructed mount with a 1-cm. cone to which the tumor was centered. The mount was attached directly to the master cone of an x-ray unit delivering 785 r tumor dose/min. Factors were 120 kv; target-skin distance, 8 cm.; HVL = 0.7 mm. Al.

Following the 5-day treatment period, the animals were observed for from 33 to 38 days (50–55 days after tumor transplantation). The animals were then sacrificed, and all tumors which had not undergone complete regression were dissected free and weighed to the nearest milligram. All abnormal tissue, no matter how small in amount or equivocal as to etiology or viability, was recorded as residual tumor, removed, and weighed.

Throughout the course of each experiment the tumors in each group were palpated at regular intervals, and a drawing of the estimated size and shape was recorded on a chart assigned to each animal.

For each experiment a group of animals labeled "sacrificed controls" was sacrificed on the day treatment of the other animals was initiated, to provide a baseline for measuring objectively the effect of treatment in the other groups.

RESULTS

The results of two representative experiments (from a total of five) are shown in Table 1. Animals that died during the course of each ex-

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periment are divided into two categories: "Dead of tumor" and "Dead of treatment." "Dead of tumor" includes those animals dying as a result of large tumor size and/or ulceration with hemorrhage, and "Dead of treatment" includes all animals not dying as a result of large tumor and presumed dead of drug toxicity. "Complete Regression" refers to those tumors which had completely disappeared by the day of sacrifice, and for the time of observation these represent "cures." The percentage of such "cures" in reference to the total number of animals in each group is indicated. The average tumor weight and standard error were determined for each group on the day of sacrifice.

X-ray.—The x-ray dose chosen for these experiments was of such a magnitude as to cause considerably less than 100 per cent permanent regression of all tumors. Because of biologic variation, the percentage of tumors "cured" with radiotherapy alone ranged among experiments from 0 to 40 per cent. Those tumors not "cured" by the x-ray treatment showed transient regression with a slow resumption of growth ("escape"). This "escape" was more delayed than that in the groups receiving only chemotherapy, as indicated by the smaller average tumor weights and the fewer animals dead of tumor.

6-MP + x-ray.—In those groups of animals receiving 6-MP + x-ray the tumors followed a

TABLE 1

RADIOTHERAPY + ADJUVANT COMBINATION CHEMOTHERAPY

(Two Representative Experiments)

		No. animals		No. tumors			
Group*	Total	Dead of tumor	Dead of treatment	Complete Regression total	Per cent "Cures"	Av. tumor wt. (mg.)	Per cent wt. change (host+tumor)
Sac. cont.	17					165±36	
Untreated cont.	19	18	0	0/19	0	1890 (1 tumor)	+15
6-AN+6-MP	19	15	0	2/19	10	428±189	+11
X-ray	19	4	0	7/19	37	173±89	+ 8
6-MP+x-ray	19	0	0	10/19	53	113±83	+ 9
6-AN+x-ray	19	1	0	8/19	42	241±123	+10
6-MP+6-AN+x-ray	19	0	0	17/19	89	8±8	+ 7
Sac. cont.	20					98±29	
Untreated cont.	19	18	0	0/19	0	2836 (1 tumor)	− 6
6-AN+6-MP	20	6	0	2/20	10	993±460	+10
X-ray	20	0	0	8/20	40	37±14	+ 6
6-MP+x-ray	21	0	0	6/21	29	110±74	+ 6
6-AN+x-ray	21	0	0	12/21	57	43±30	+ 6
6-MP+6-AN+x-ray	18	0	0	12/18	67	18±16	+ 8

* Sac. cont. = animals sacrificed on day injections begun to other groups; all treatment was begun 17 days after implantation of mammary adenocarcinoma 755 to C57BL mice. After 5 days of treatment the animals were observed for 33–38 days before termination. 6-AN = 6-Aminonicotinamide; 6-MP = 6-mercaptopurine.

Untreated.—As can be noted in Table 1, nearly all the untreated animals were dead of tumor by the end of the 33–38-day observation period (50–55 days after tumor implantation). The tumors from the few surviving animals were large, as can be seen under "Av. tumor wt.," and no tumors regressed spontaneously.

6-MP + 6-AN.—Palpation of the tumors in those animals receiving only chemotherapy (i.e., 6-MP + 6-AN) indicated, in general, that tumor growth was virtually stopped for the 5-day treatment period and for approximately 5 days thereafter. At the end of this lag period the tumors rapidly "escaped" and grew to a size sufficient to kill most of the animals before the day of sacrifice. Although an occasional tumor "cure" (0–10 per cent) was noted in animals surviving to the end of the observation period, the great majority had large tumors.

pattern similar to that observed with x-ray alone. The percentage of tumors "cured" with 6-MP + x-ray (25 per cent–53 per cent), while slightly higher than with x-ray alone (0–40 per cent), was of no significant magnitude.

6-AN + x-ray.—The tumors in the groups of animals receiving 6-AN + x-ray also followed a pattern similar to that observed with x-ray alone. The percentage of tumors "cured" with 6-AN + x-ray (21 per cent–57 per cent) was slightly but not significantly higher than that with x-ray alone (0–40 per cent).

6-MP + 6-AN + x-ray.—A marked increase in tumor damage resulting from the administration of 6-MP + 6-AN + x-ray, over that of x-ray alone, both in per cent tumor "cures" (37–89 per cent) and in average tumor weight, was seen in every experiment. In addition, the percentage of tumors "cured" was significantly greater with both drugs

plus x-ray than with either drug alone plus x-ray. According to palpation, none of the tumors in these groups of animals receiving the combined chemotherapy and x-ray had "escaped" by the end of the observation period, whereas there were some "escapes" in the groups receiving only one drug + x-ray. This may be more objectively ascertained by comparing the average tumor weights of the groups with those of their respective "sacrificed controls."

A comparison of the average percentage of "cures" for all groups in the five experiments is graphically represented in Chart 1.

DISCUSSION

It is apparent from the data presented that minimal chemotherapy acted as a powerful adjuvant to radiotherapy in producing tumor damage. The average percentage of tumors "cured" was increased from 27 per cent, with x-ray alone, to 63 per cent with 6-MP + 6-AN + x-ray (Chart 1). This markedly increased antineoplastic effect was accomplished with only five injections of two chemical agents, each of which is merely carcinostatic when administered alone to animals bearing well established (14-day-old) 755 tumors (6, 7).

Therefore, it must be emphasized that the recorded antineoplastic effects are not necessarily the best obtainable with a combination of radiotherapy and either or both of these drugs. For example, unpublished studies in this laboratory have indicated that 6-AN alone can significantly augment radiation tumor damage under different experimental conditions. In addition, x-ray alone, at a dose approximately 20 per cent–30 per cent greater than that employed here, would be in the range expected to produce a very high percentage of 755 tumor "cures." Such an increase in radiation dosage, however, would mask any chemotherapeutic augmentation. (It would also be inconsistent with the clinical situation in which normal human tissue tolerance prohibits appreciable increase in tumor dose beyond established radiation regimens.) In the present experiments, therefore, minimal doses of 6-AN, 6-MP, and x-ray were deliberately chosen to explore further our previously published concepts (4–6, 8–12) that there is merit in combining therapy at a multiple level.

Many variations of the combined therapy administration, such as doses, duration, sequence, additional drugs, etc., remain to be investigated. Studies of the possibilities inherent in such alterations are now in progress.

It is felt that the documented results recommend similar methodology for clinical evaluation of certain potential cancer chemotherapeutic agents. Of practical import, the data suggest two specific chemical agents to be evaluated in conjunction with radiotherapy in cancer patients.

SUMMARY

Marked augmentation of radiotherapeutic "cure" rates upon mammary adenocarcinoma 755 was obtained by combined treatment with two chemical agents (6-aminonicotinamide + 6-mercaptopurine) and x-ray.

The data recommend similar methodology—radiotherapy + adjuvant chemotherapy—for clinical evaluation of these two agents upon solid tumors.

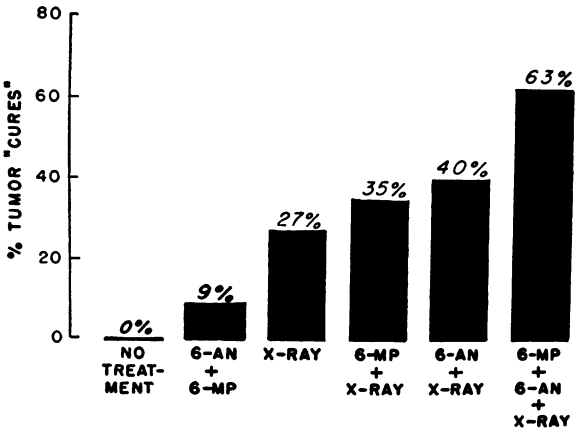

CHART 1.—Augmented "cure" rates obtained with multitherapy as indicated. Summary of five experiments.

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