Radiopotentiating Action of 1-β-D-Arabinofuranosylcytosine in a

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1-β-D-Arabinofuranosylcytosine [cytosine arabinoside (ara-C)] has proved effective in inducing remissions in acute leukemia and lymphoma (1–5) and in polycythemia vera (6). Some anticancer activity has also been reported in certain solid neoplasms (7, 8). The finding that ara-C has a radiosensitizing action in *Micrococcus radiodurans* (9) raises, especially because of its widespread clinical use, the question of whether this agent might have radiopotentiating properties in an experimental tumor system *in vivo*.

Materials and Methods. The tumor chosen for these experiments is the human colonic carcinoma *GW-39*, serially transplantable in unconditioned hamsters (10). This neoplasm is almost exclusively composed of mucin-secreting signet-ring tumor cells which have been found to be relatively refractive to either ara-C or X-radiation (11–13). The procedures used to determine the radiation-modifying activity of drugs in GW-39 tumors growing in hamster cheek pouches have been described in detail (13). Briefly, GW-39 cheek pouch tumors were transplanted to both cheek pouches of male and female golden hamsters (*Mesocricetus auratus*) weighing 50–70 g. The animals were thereafter randomized into groups of 10–15; one group served as controls, receiving ip therapy with the drug vehicle and manipulation of their cheek pouches, while the remaining groups received either single or combination therapy according to the following schedules. In the irradiated groups, X-rays were delivered to the everted left cheek pouch tumor by a Siemens Dermopan apparatus (29 kV tube voltage, 25 mA output, 0.3 mm Al filtration, focus-tumor distance of 15 cm, and a rate in air of 841 R/min) only on Day 10 post transplantation; the rest of the animal was shielded with lead strips. Ara-C pure base, made available in its commercial form by Heinrich Mack Nachf., Illertissen, Bavaria, was prepared fresh daily in physiological saline so that an amount of 1 ml/100 g body weight was administered ip daily as a single injection, or in three divided doses, for 7 consecutive days (Days 7 through 13 post-grafting). Tumor growth-inhibition was determined by comparing mean increase in tumor size, based upon the initial tumor measurement at 5 days post transplantation, of treated vs controls. Tumor size was calculated by measuring the tumors' length, width, and depth with a small caliper both before and at several intervals during and after therapy. These procedures and the reliability of the calculations obtained have been considered in detail elsewhere (11, 14).

Results. Radiopotentiation is here defined as any significant improvement above the expected arithmetic sum of each modality's own range of tumor growth-inhibition without a concomitant increase in host toxicity. As such, no differentiation between the terms "potentiation," "synergism," "augmentation," or "enhancement" will be attempted.

At the maximal tolerated single daily dose of ara-C in the hamster, which we have determined to be about 24 mg/kg ip (11, 12), no unequivocal radiopotentiating effects of ara-C could be ascertained. Reducing this dose of ara-C by one-half, however, does result in a synergistic antitumor effect for the combination of ara-C and 300 R X-rays, as demonstrated in Fig. 1. This is particularly striking when each parameter alone is at its minimally effective dose. Whereas the maximum tumor growth-inhibition obtained for this com-

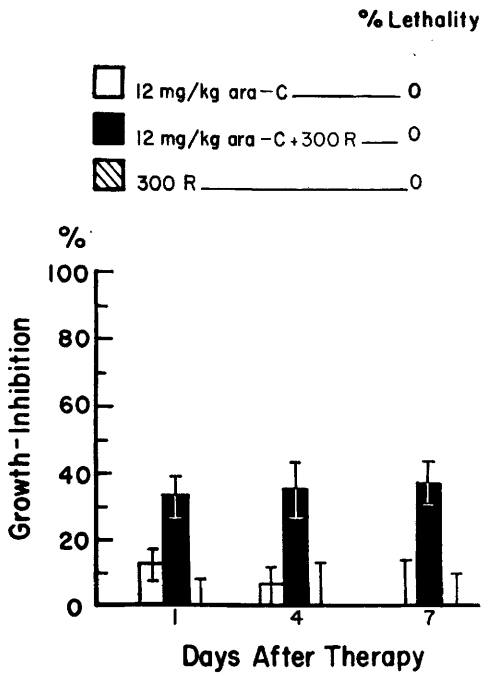

FIG. 1. Growth-inhibition of GW-39 tumors at 1, 4, and 7 days post therapy with ara-C (12 mg/kg × 7), X-rays (300 R × 1), and both combined. Vertical bars represent SE of the mean.

bination was 40%, this could be increased to an impressive 70% by merely altering ara-C's dosage schedule to 4 mg/kg three times daily (Fig. 2). The improved efficacy of a divided dose schedule is again reflected in the tumor-inhibitory effects of ara-C alone, yielding a growth-inhibition equivalent to that of the combination therapy of the preceding experiment.

Discussion. It appears from these results that potentiation of low, nontumoricidal levels of X-radiation can be accomplished with low doses of ara-C in an *in vivo* tumor system relatively refractive to each modality alone. It is not clear why low doses of both cytotoxic measures in combination yield clearer evidence of synergism than do higher doses, but this might certainly be more related to a technical problem in demonstrating such effects than to something inherent to the possible potentiation of each agent's biochemical lesion(s). As we have discussed at greater length elsewhere (13), one must nevertheless also consider such factors as (1) summation of sublethal actions of each parameter, (2) interference with cell recovery mechanisms and/or (3) catabolism of the drug, and (4) immunological participation of the host, particularly in a system which is xenogeneic. However, there is no evidence, with regard to the latter possibility, that any host defense mechanisms are evoked by the GW-39 tumor growing in the hamster cheek pouch.

It does not appear, based upon other combinations of drugs and X-rays tested to date, that the radiopotentiation witnessed here is unique for ara-C or other agents with similar mechanisms of action. On the contrary, we have found that a number of drugs examined from the major groups of cancerostatic agents show, but only at low dosage levels, synergistic effects when combined with a low dose of X-rays (13). This suggests that the synergistic efficacy of both therapeutic measures in combination is perhaps more a function of their respective dosages than their individual mechanisms of action. In

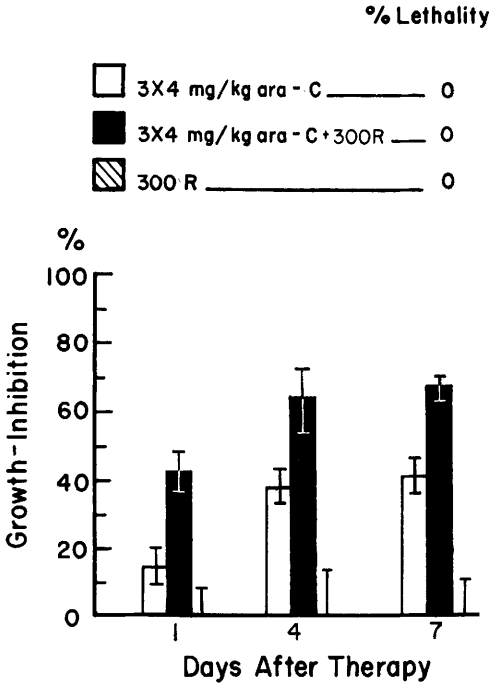

FIG. 2. Growth-inhibiting effects of ara-C [(3 × 4 mg/kg) × 7], 300 R X-rays, and both combined against GW-39 tumors in the hamster cheek pouch.

other words, whereas it is commonly held that certain drugs are more radiopotentiating than others, it may well be that any agent can, at a particular dosage level, exhibit such properties. At lower doses, therapeutic synergism would not appear to be a specific effect of the combination in question, but perhaps attributable to more general toxic actions. However, this has not proved completely true for the divided drug schedule of ara-C, which yielded maximal antitumor effects both for ara-C alone and ara-C combined with X-radiation. An analysis of the cell cycle kinetics of this tumor in relation to different dose schedules of radiation and drug therapy should help resolve this question.

Whereas it is still premature to advocate the use of ara-C combined with radiation in clinical therapy, the radiopotentiating effects of ara-C shown here do provoke the assessment of this therapeutic combination in other experimental tumor systems.

Summary. Low, nontumoricidal, single daily doses of ara-C had radiopotentiating effects when combined with 300 R X-rays in the GW-39 human colonic carcinoma growing in the cheek pouch of unconditioned golden hamsters. At the same total dosage (12 mg/kg day), a divided daily dose schedule of ara-C proved more effective both when administered alone and in combination with X-radiation.

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