Sarcoma 180 Inhibition by Combinations of 6-Thioguanine and Uracil Mustard

Abstract. Combinations of 6-thioguanine and uracil mustard produced greater inhibition of the growth of sarcoma 180 than that equivalent to the sum of the inhibitory effects caused by the optimum levels of the individual drugs. This potentiation was accomplished without marked weight loss by the host. Some possible biochemical mechanisms responsible for the drug synergy are discussed.

6-Thioguanine is one of several chemical agents capable of sensitizing cells to the inhibitory action of both ultraviolet and x-irradiation (*1*). Since some biochemical and biological effects of alkylating agents resemble those of ionizing radiation in a number of respects (*2*), although it is probable that these common events are the end products of different modes of action (*3*), we measured the ability of thioguanine to sensitize sarcoma 180 ascites tumor cells to the growth-inhibitory action of the alkylating agent uracil mustard [5-bis-(2-chloroethyl) aminouracil].

Female Ha/ICR Swiss mice, 9 to 11 weeks of age (*4*), were each implanted with approximately 4×10^6 tumor cells in a manner previously described (*5*). Therapy was initiated 24 hours after tumor implantation and continued for six consecutive days. Therapeutic efficacy was determined by three criteria: (i) the prolongation of survival time afforded by drug treatments, (ii) the number of 50-day survivors, and (iii) the number of regressions of tumor growth (*5*). The percentage change in weight from onset to termination of therapy was used as an indication of toxicity.

Table 1 indicates that thioguanine produced only a slight prolongation of survival time; maximum effective doses were 1.0 to 2.0 mg/kg of body weight. With the maximum effective level of uracil mustard, 0.5 mg/kg, 20 percent of the tumor-bearing animals survived 50 days; each of these survivors was subjected to autopsy at 100 days and found to be free of neoplastic growth. Increasing the level of uracil mustard to 0.75 mg/kg resulted in pronounced weight loss (18.4 percent) with no apparent gain in carcinolytic effectiveness. Several combinations of the two agents possessed tumor-inhibitory properties greater than those equivalent to the sum of the maximally effective levels of the individual drugs. The most effective therapy consisted of 1.0 mg of thioguanine per kilogram in combination with 0.25 mg of uracil mustard per kilogram; 70 percent of the tumor-bearing animals that received this regimen survived 50 days, and 50 percent of the treated animals were found to be tumor-free. Toxicity, as measured by a decreased body weight, was only 5 percent and therefore was considered minimal. Evidence for potentiation with this combination of agents has been obtained in animals bearing either the Ehrlich ascites carcinoma or subcutaneous implants of hepatoma 134 (*6*).

The ability of combinations of thioguanine and uracil mustard to produce an enhanced inhibition of tumor growth appears to be the result of the inhibition of complementary biochemical processes, rather than of the superimposition of two unrelated toxic events. Thus, substitution of the daily simultaneous administration of the two agents by a regimen in which uracil mustard was administered 12 hours prior to the administration of 6-thioguanine resulted in a decrease in the synergistic activity. Under both of these dosage schedules, unrelated additive toxicity should be expected; the necessity for simultaneous administration of the two drugs indicates that time-dependent metabolic alterations occur that are responsible for the synergic tumor-inhibitory properties. In addition, combination of uracil mustard with either 8-azaguanine or azaserine, both potent inhibitors of purine nucleotide biosynthesis, in the therapy of mice bearing sarcoma 180 ascites cells, did not result in an inhibition of tumor growth superior to that produced by uracil mustard alone (*6*); this finding indicated further that nonspecific additive cytotoxicity is not responsible for the potentiated tumor-inhibitory effects of the combination of thioguanine and uracil mustard.

The biochemical mechanism of the potentiation is unknown; it is conceivable, however, that the effectiveness of the drug combination is related to the ability of uracil mustard to alkylate the N-7 position of the guanine portions of the polynucleotides (*7*). During a subsequent repair process, the thioguanine or a metabolite containing thioguanine could inhibit the formation of new molecules of guanine nucleotides (*8*) or could replace alkylated guanine residues lost from deoxyribonucleic acid (DNA) (*7*). In this manner, a DNA would be produced which contained a relatively large quantity of the purine analog. Incorporation of thioguanine into DNA has been postulated both as a mechanism of tumor inhibition (*9*) and as a mechanism of sensitization to ultraviolet and x-irradiation (*1*). However, since both 5-iodo-2′-deoxyuridine, which is incorporated into DNA, and 5-fluorouracil, which is not incorporated into DNA, are capable of enhancing the carcinostatic action of uracil mustard (*6*), this mechanism would appear to be distinct from that involved in sensitization to ultraviolet or to x-irradiation. Furthermore, 5-fluorouracil does not increase the effec-

Table 1. Synergic response of sarcoma 180 ascites tumor-bearing mice to combination chemotherapy with 6-thioguanine and uracil mustard. Mice were treated by intraperitoneal injection for six consecutive days beginning 24 hours after implantation of ascites cells. Combination treatments were given simultaneously. Mice surviving over 50 days and tumor-free animals were calculated as 50-day survivors.

Daily dosage (mg/kg)		Av. change in body wt., days 0 to 7 (%)	Av. survival (days)	No. of 50-day survivors	No. of regressions
Thioguanine	Uracil mustard				
0	0	+15.5	13.6	0/20	0/20
0.5	0	+25.3	16.1	0/10	0/10
1.0	0	+21.2	18.8	0/10	0/10
2.0	0	+16.0	19.5	0/10	0/10
4.0	0	+13.4	13.1	0/10	0/10
0	0.125	+21.6	18.1	0/20	0/20
0	0.25	— 2.9	25.8	3/25	1/25
0	0.50	—13.0	35.6	4/20	4/20
0	0.75	—18.4	32.0	1/5	0/5
0.5	0.125	+18.8	24.1	2/10	1/10
0.5	0.25	0	38.8	4/10	4/10
0.5	0.5	— 6.7	44.0	5/10	3/10
1.0	0.125	+ 4.8	35.2	4/10	3/10
1.0	0.25	— 4.8	45.3	7/10	5/10
1.0	0.50	—16.1	39.0	4/10	2/10

tiveness of irradiation (*1*, *10*). Nevertheless, it is conceivable that a general mechanism of cellular sensitization to the alkylating agents is involved which is common to all of the effective antimetabolites. One possibility that would be consistent with all the effective drug combinations implies a simultaneous action on DNA; uracil mustard would alkylate the polynucleotide molecules and the various antimetabolites each would limit repair processes by producing different enzymic blockades that decrease the supply of essential metabolites (*11*).

BARBARA A. BOOTH
ALAN C. SARTORELLI
Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut

References and Notes

1. H. S. Kaplan, K. C. Smith, P. Tomlin, *Nature* **190**, 794 (1961).
2. E. L. Powers and J. H. Pomeroy, *Ann. N.Y. Acad. Sci.* **68**, 702 (1958); G. M. Timmis, *Advan. Cancer Res.* **6**, 369 (1961).
3. P. Alexander, *Advan. Cancer Res.* **2**, 5 (1954).
4. From the A. R. Schmidt Co., Madison, Wis.
5. A. C. Sartorelli, P. F. Kruse, Jr., B. A. Booth, E. J. Schoolar, Jr., *Cancer Res.* **20**, part 2, 495 (1960).
6. B. A. Booth and A. C. Sartorelli, unpublished observations.
7. P. Brookes and P. D. Lawley, *Biochem. J.* **80**, 496 (1961).
8. A. C. Sartorelli and G. A. LePage, *Cancer Res.* **18**, 1329 (1958).
9. G. A. LePage, *ibid.* **20**, 403 (1960).
10. W. Szybalski, *Animal Cell Inform. Serv.* **1**, 5 (1959).
11. Supported by National Cancer Institute grant CY-2817. We are indebted to Mrs. Shelly F. Williams and to Richard A. Lopes for assistance, and the Upjohn Company, Kalamazoo, Mich., for uracil mustard.

2 August 1962

Maintenance of Globulin Levels in X-irradiated Rabbits after Immunization

Abstract. Rabbits were injected with antigen 24 hours after x-irradiation. Antibody titers were correlated with relative changes in gamma-beta globulin levels determined electrophoretically. Irradiated, immunized rabbits did not form detectable antibodies but had significantly greater globulin levels than non-immunized, irradiated controls. This relative difference occurred at the time nonirradiated, immunized rabbits were producing primary antibody.

A primary antibody response can be prevented or delayed by x-rays (*1*). Prevention or partial alleviation of the radiation effect has been achieved by injecting nucleic acid derivatives from a variety of sources at the time of antigen injection (*2*). It was suggested that such substances provide replacements for radiation-damaged materials which normally mediate the antigenic stimulus.

The effect of radiation on antibody formation might result from elimination of antibody-forming cells by direct destruction or from prevention of cell division through interference with nucleic acid synthesis.

Alternatively, should antibody synthesis derive from a process of information transfer by nucleoprotein with or without antigenic fragments (*3*) the effect of radiation could stem from x-ray-induced nucleic acid disorganization leading to aberrant intermediates capable of participation in cellular metabolism and, possibly, to faulty transfer or utilization of the antigenic information. Protein produced in response to such aberrant stimuli might not be identifiable by reaction with the antigen stimulating its production. Antibody formation would appear to have been prevented; yet globulin synthesized in response to antigen would be present.

Male New Zealand rabbits were given a single intravenous injection of bovine albumin (BSA, 10 or 15 mg) (*4*), in rabbit hemoglobin particle adjuvant (*5*). Antigen was injected 24 hours after the rabbits were x-irradiated. The whole body was exposed to 400 r generated at 250 kv and 15 ma by a Picker x-ray machine. The vertical beam was filtered by 1.0 mm of aluminum and 0.5 mm of copper. The half value layer of the beam was 3.0 mm of copper. The dose was measured in air to the center of the box.

Blood samples were collected periodically. Serum proteins were separated with the Spinco model R paper electrophoresis system. Paper strips were analyzed in the Spinco Analytrol. Relative concentrations of individual serum fractions were obtained by drawing verticals and relating the area under each section of the photometrically obtained protein curve to the total curve (*6*). Total serum protein was determined by the biuret method (*7*).

Occurrence of an immune response was judged in two ways. The immune catabolism technique (*8*) was employed with enough I^{131}-labeled bovine serum albumin (*9*) to contain approximately 3×10^6 count/min. Antibodies were also detected by the Farr ammonium sulfate procedure (*10*). The antigen binding capacity was taken as the percentage of 0.05 μg of BSA-I^{131} bound by a 1:5 dilution of serum. With these procedures primary antibody was detected in normal animals between 7 and 10 days after injection.

Similar experiments were performed on nonirradiated, immunized and on irradiated, nonimmunized rabbits. Ten normal rabbits were included as bleeding controls. Comparisons were made between these groups and the irradiated, immunized group.

Figure 1 shows the average primary response to antigen by immune elimination and by Farr techniques. The irradiated, immunized group did not produce antibodies. They showed a constant rate of antigen elimination and an unchanged antigen binding capacity. The nonirradiated rabbits showed an increased rate of elimination between the 7th and 8th days and an increased binding capacity 1 or 2 days later. Eleven of the immunized, irradiated rabbits were bled again 20 days after injection.

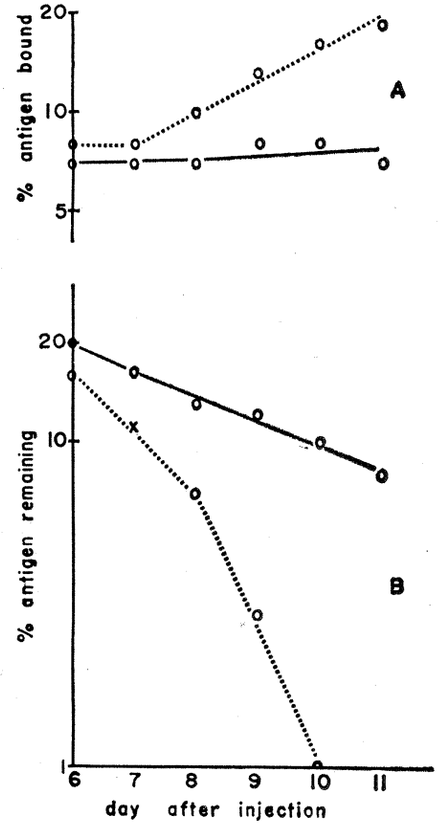

Fig. 1. Semilogarithmic plot of response to bovine albumin by irradiated, immunized rabbits (solid line) and non-irradiated, immunized rabbits (dotted line). *A*, Mean antigen binding capacity expressed as percentage of antigen bound by a 1:5 serum dilution. *B*, Mean disappearance of circulating antigen expressed as percentage of initial activity. Minimum of ten rabbits per point (*X* represents nine rabbits).