Combination Chemotherapy with 8-Azaguanine and Sex Hormones on a Mouse Mammary Carcinoma*

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The possibility that more effective cancer chemotherapy may be achieved by treatment with combinations of two or more agents has received increasing attention recently. Skipper (8) has reported an antileukemic synergism between urethan and methylbis(2-chloroethyl)amine. Stoerk (9) presented evidence for increased growth retardation of lymphosarcoma implants in animals on a pyridoxine-deficient diet after the administration of either cortisone or methyl testosterone with desoxypyridoxine. Shapiro and Gellhorn (7) demonstrated that a transplantable carcinoma in mice may be inhibited to a significantly greater extent when the carcinostatic agent 8-azaguanine is combined with desoxypyridoxine, folic acid, or 7-methyl folic acid. Both Law (3) and Goldin and his collaborators (2) have observed additive effects upon an acute lymphoid leukemia in mice with a combination of two antimetabolites, aminopterin and 8-azaguanine.

These reports of synergism between chemotherapeutic agents provide data of experimental and potential clinical interest. In view of these findings, it was decided that the catalytic role in biochemical reactions attributed to hormones warranted their evaluation with anti-cancer agents. The successful clinical use of the sex hormones in certain malignant growths prompted the selection of these compounds for initial study. The present report concerns the findings on a transplantable carcinoma when treated with 8-azaguanine in combination with either testosterone or stilbestrol, or both.

An unexpected result of these studies was the demonstration that stilbestrol alone inhibited the growth of a transplantable mammary carcinoma. A review of the literature revealed that no other laboratory tumor has been reported susceptible to stilbestrol therapy. It was further demonstrated that the combination of 8-azaguanine and stilbestrol had a striking carcinostatic effect upon the mammary adenocarcinoma.

METHODS

The neoplasm employed in this study was the 755 tumor, a transplantable mammary adenocarcinoma, grown in C57BL mice. The consistent carcinostatic action of 8-azaguanine upon this tumor has been previously recorded (1).

The mice were maintained in plastic cages in an air-conditioned constant-temperature room (74° F.) and were offered Rockland pellets and water ad libitum. Mice, 2–3 months old and weighing 18–25 gm., were given inoculations of tumor fragments in the axillary region by the usual trocar method. Daily therapy was initiated at various intervals after transplantation, as indicated in the tabular summaries, and continued until the experiment was completed. Objective evidence for the presence or absence of anti-neoplastic effect was based on the wet weights of the tumors determined to the nearest milligram. In making a statistical evaluation of the significance of the difference between two mean tumor weights (m_1 and m_2), the results have been considered beyond chance variation when

$$\frac{m_1 - m_2}{\sqrt{(\sigma m_1)^2 + (\sigma m_2)^2}} = 2.5 \text{ or greater .}$$

The guanine analog, 8-azaguanine,[1] was dissolved in 0.1 N NaOH as previously described (1), and injected intraperitoneally. Diethylstilbestrol[1] was dissolved in sesame oil and injected intramuscularly. Both aqueous testosterone[1] and testosterone proprionate in oil[1] were also administered intramuscularly.

RESULTS

The administration of testosterone did not affect the growth of the 755 tumor, as may be concluded from the findings recorded in Table 1. With the exception of experiment 19A, the results of the remaining four experiments, summarized in Table 2, reveal that the combination of 8-azaguanine and testosterone produces no greater effect on the tumor's growth than can be obtained with 8-azaguanine alone. The marked weight gain in the testosterone-treated mice is to be noted.

Table 3 summarizes the results of experiments

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with stilbestrol, administered either alone or in combination with testosterone. In every experiment stilbestrol significantly inhibited the growth of this mammary mouse carcinoma. As is common in animals treated with stilbestrol, a moderate degree of weight loss is obtained. This is especially apparent in the stilbestrol-treated mice in experiment 15B. However, these animals received a larger dose of stilbestrol (50 mg/kg) than was given to mice in the other experiments (10 mg/kg). This weight loss is either completely prevented or minimized to insignificant levels by combining therapy with testosterone. This prevention of the toxic picture produced by stilbestrol on the weight of the treated animals is accomplished without blocking the growth-retarding effect of stilbestrol upon the tumor.

The marked carcinostatic activity afforded by the combination of 8-azaguanine plus stilbestrol is presented in Table 4. With the exception of experiment 26, the addition of testosterone to this combination results in minimal weight loss with no reduction in carcinostatic activity. The results of these experiments recording enhanced carcinostasis due to the combination of stilbestrol with 8-azaguanine are statistically significant.

TABLE 1

EFFECT OF TESTOSTERONE ON THE 755 CARCINOMA IN C57BL MICE

Exp. no.	Sex	Group	Mean tumor wt. (mg.)	No. animals dead/total	Per cent change in body wt.
7	♀	Controls	1,087	0/30	+13
		Testosterone	942	0/30	+49
12A	♂	Controls	1,041	0/20	+14
		Testosterone	1,023	1/20	+20
15A	♂	Controls	1,041	1/19	+ 4
		Testosterone	888	2/20	+12
15B	♂	Controls	948	0/20	+ 7
		Testosterone	1,023	0/19	+14

NOTE: The experiments vary in the time interval after transplantation at which therapy was begun (from 3 to 5 days) and in the duration of tumor growth (from 17 to 20 days).

Either aqueous testosterone (7, 12A) or testosterone proprionate in oil (15A, 15B) was administered intramuscularly daily. Experiment 7 received 25 mg/kg; experiments 12A, 15A, and 15B received 50 mg/kg of body weight.

TABLE 2

EFFECT OF TESTOSTERONE IN COMBINATION WITH 8-AZAGUANINE ON THE 755 CARCINOMA IN C57 MICE

Exp. no.	Sex	Group	Mean tumor wt. (mg.)	No. animals dead/total	Per cent change in body wt.
14B	♂	Azaguanine	232	0/28	− 5
		Aza.+Test.	235	0/20	+ 1
15A	♀	Azaguanine	180	1/20	− 2
		Aza.+Test.	204	0/20	+ 1
15B	♂	Azaguanine	188	1/19	− 2
		Aza.+Test.	231	2/20	+23
19A	♂	Azaguanine	944	1/20	+11
		Aza.+Test.	388	1/20	+11
19B	♀	Azaguanine	471	0/19	+ 8
		Aza.+Test.	437	0/20	+14

NOTE: The experiments vary in the time interval after transplantation at which therapy was begun (from 3 to 9 days) and in the duration of tumor growth (from 20 to 24 days).

8-Azaguanine was administered daily at 50 mg/kg intraperitoneally; testosterone proprionate in oil at 50 mg/kg intramuscularly daily.

DISCUSSION

The mechanism by which testosterone prevents excessive weight loss produced by stilbestrol is not understood. It is possible that this is not an antagonistic reaction between the two hormones. The known anabolic effect of testosterone upon protein metabolism may result in a weight gain

TABLE 3

EFFECT OF DIETHYLSTILBESTROL, ALONE AND IN COMBINATION WITH TESTOSTERONE, ON THE 755 CARCINOMA IN C57BL MICE

Exp. no.	Sex	Group	Mean tumor wt. (mg.)	No. animals dead/total	Per cent change in body wt.
	♂	Controls	948	0/20	+ 4
15B		Stilbestrol	404	0/20	−19
		Stilb.+Test.	347	8/20	− 5
	♀	Controls	1,534	0/15	+ 6
19C		Stilbestrol	980	4/15	− 4
		Stilb.+Test.	628	5/17	+ 2
	♀	Controls	907	1/20	+ 8
21		Stilbestrol	531	0/20	− 8
		Stilb.+Test.	696	4/20	+ 2
25	♀	Controls	910	0/19	+ 7
		Stilbestrol	206	0/20	−10

NOTE: The experiments vary in the time interval after transplantation at which therapy was begun (from 3 to 9 days) and in the duration of tumor growth (from 17 to 22 days).

Both hormones were dissolved in oil and administered intramuscularly daily. Testosterone was given at 50 mg/kg; diethylstilbestrol at 50 mg/kg (×5) and 25 mg. (×5) in experiment 15B and at 10 mg/kg in the other experiments.

TABLE 4

EFFECT OF COMBINATION OF 8-AZAGUANINE, DIETHYLSTILBESTROL AND TESTOSTERONE ON THE 755 CARCINOMA IN C57BL MICE

Exp. no.	Sex	Group	Mean tumor wt. (mg.)	No. animals dead/total	Per cent change in body wt.
	♂	Azaguanine	944	1/20	+11
19A		Aza.+Stilb.	77	2/20	− 9
		Aza.+Stilb.+Test.	52	4/20	− 1
	♀	Azaguanine	471	0/19	+ 8
19B		Aza.+Stilb.	59	3/20	−14
		Aza.+Stilb.+Test.	118	2/20	+ 2
	♀	Azaguanine	296	0/25	+ 5
22		Aza.+Stilb.	102	0/25	− 5
		Aza.+Stilb.+Test.	152	3/25	0
	♂	Azaguanine	576	1/20	+ 3
26		Aza.+Stilb.	211	1/20	− 5
		Aza.+Stilb.+Test.	171	1/20	− 8

NOTE: The experiments vary in the time interval after transplantation at which therapy was begun (from 7 to 10 days) and in the duration of tumor growth (from 20 to 24 days).

Diethylstilbestrol (in oil) was given daily intramuscularly at 10 mg/kg; testosterone proprionate (in oil) was administered daily intramuscularly at 50 mg/kg; and 8-azaguanine at 50 mg/kg intraperitoneally daily.

which effectively overcomes the weight loss produced by stilbestrol.

The beneficial response of certain human malignancies to stilbestrol therapy has warranted numerous studies to find a laboratory neoplasm which would similarly respond. These attempts have thus far been unsuccessful. Negative results have also been obtained by many workers using a variety of estrogens. However, Nathanson and Salter (5, 6) reported that Sarcoma 180 was inhibited by estrone when the tumor was grown in C57BL mice but not when the tumors were borne by Bagg albino, strain A mice, unless the host's resistance against the neoplasm was increased by preliminary "immunization." The possibility that the proper genetic background is the only factor responsible for the effectiveness of stilbestrol against the 755 carcinoma seems doubtful in view of the report by Ludford and Dmochowski (4). These investigators studied the action of diethylstilbestrol upon ten different transplantable mouse tumors in mice of four inbred strains. It appears pertinent to note that their negative results encompass two carcinomas carried in C57BL mice. Their results lend circumstantial support to the possibility that these reported findings may be specific rather than nonspecific.

SUMMARY

1. Diethylstilbestrol inhibits the growth of the 755 mammary adenocarcinoma in C57BL mice.

2. The combination of 8-azaguanine and stilbestrol has a striking carcinostatic effect upon the 755 tumor.

3. Weight loss induced by stilbestrol in the experimental animals is reduced to insignificant levels by the addition of testosterone, without affecting carcinostatic activity.

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