

Effect of Hydroxyurea on Growth of a Transplantable Mouse Mammary Adenocarcinoma

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Summary

Growth of mammary adenocarcinoma H2712 is inhibited by hydroxyurea. This compound was effective by daily, intermittent, or single s.c. injection. It was also effective when administered directly into the tumor.

Hydroxyurea is most efficacious when administered prior to the onset of rapid growth.

Simultaneous administration of hormones with antitumor activity in this system did not augment the effects of hydroxyurea.

Introduction

Hydroxyurea (Hydrea—Squibb) has been shown to possess antineoplastic activity in a number of animal tumor systems (2, 7, 11, 12, 15, 16), as well as in man (8, 9, 17). Preliminary studies demonstrated that this compound can retard the growth of the mammary adenocarcinoma H2712 (11). The present investigation was undertaken to explore the effect of hydroxyurea on the growth of this tumor under different conditions of dose, onset of treatment, frequency of treatment, and combined therapy.

Materials and Methods

The mammary adenocarcinoma H2712 was grown in adult female C3H/HeJ mice. At 7–8 days postimplantation of the tumor, these donor mice were sacrificed by cervical dislocation and the tumor area swabbed with 70% ethyl alcohol. Tumors which were well-developed but not necrotic were selected for transplantation. Each tumor was excised and placed in a Petri dish containing sterile saline solution. The tissue was then minced into pieces small enough to fit into a 17-gauge trochar. Transplantation was made by trochar technic into adult male and female C3H/HeJ mice. The tumor piece was placed on the right medioventral portion of the thorax between the ribs and the skin. Aseptic technic was followed during the transplantation procedure.

Dose groups of 8–14 mice, weighing 20–22 gm each, were employed. These animals were provided with ground Rockland mouse chow and water *ad libitum*. Hydroxyurea and human chorionic gonadotropin (Follutein—Squibb) were dissolved in water; testosterone propionate and 17 α -hydroxyprogesterone caproate (Delalutin—Squibb) were dissolved in sesame oil.

Injections were via the s.c. route and administered in areas removed from that of the tumor implantation site; an exception to this was the study involving intratumor injections.

Results

The mammary adenocarcinoma (H2712) was originally obtained from the Jackson Memorial Laboratories at Bar Harbor and transplanted in C3H/HeJ mice for a number of transplant generations (approximately 50). Growth of H2712 was very rapid (Chart 1). It was palpable 3–4 days postimplantation, and rapid growth started 3 days later. At 11 or 12 days after tumor implantation, the tumor reached a maximum weight of 3–4 gm. Also at this time some animals died, and by 15–20 days all mice had succumbed. Tumor growth inhibited body (carcass) growth. A loss in body weight was first noted on Day 8 after tumor implantation. When tumor weight was at its maximum, the loss in body weight was also at its maximum, averaging 2–3 gm.

Daily s.c. administration of 5 mg of hydroxyurea (approximately 250 mg/kg) starting on the day of tumor implantation reduced tumor growth approximately 90% when this tissue was weighed on Day 10. A dose of 1 mg/day was almost as effective in inhibiting H2712 growth. On Day 10 tumor growth was inhibited in female and male animals by 75% and 80%, respectively. The 0.2-mg dose was much less effective. Treatment with hydroxyurea not only inhibited tumor growth, but also prevented body weight loss. On Day 10 after tumor implantation in the absence of treatment, the average carcass weight was 2.3 gm less than on Day 0. With the daily administration of 0.2, 1.0, and 5.0 mg of hydroxyurea, the changes in carcass weight on Day 10 were -0.5, +0.1, and +4.2, respectively.

Testosterone propionate, 17 α -hydroxyprogesterone caproate, and human chorionic gonadotrophin inhibit the growth of this tumor (10, 13); therefore, each of these hormonal substances was administered concomitantly with hydroxyurea to determine whether additive or augmentative activity could be obtained. It is interesting that not only were such combinations not additive, but in every case the resultant inhibition of tumor growth in male and female mice was less than that induced by hydroxyurea (1 mg/day) alone.

Administration of a toxic agent on the day of tumor implantation might possibly retard growth by preventing an adequate "take" of the injected tissue and therefore, present an inaccurate index of activity. A study was therefore made of the effect of hydroxyurea (1 mg/day) on tumor weight when treatment was

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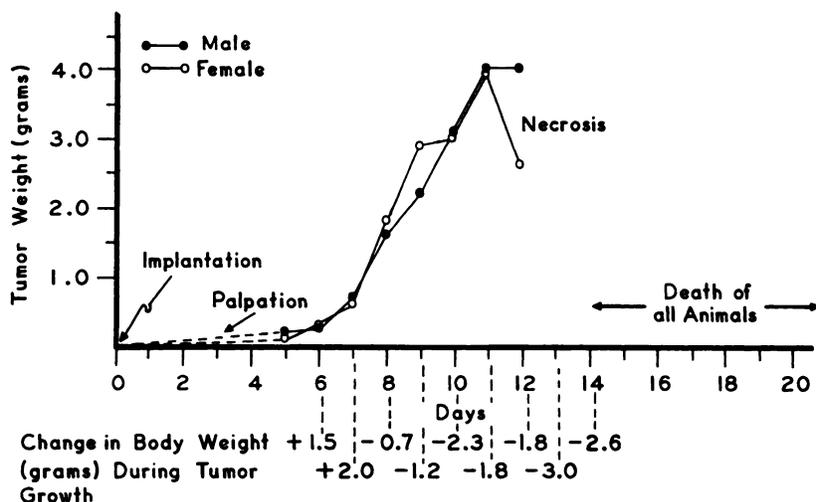


CHART 1. Growth of the mammary adenocarcinoma (H2712) and effects of such growth on body weight in male and female C3H HeJ mice.

TABLE 1
EFFECT OF HYDROXYUREA ON THE MAMMARY ADENOCARCINOMA H2712 IN C3H/HeJ MICE

DAILY TREATMENT	DAY OF INITIAL TREATMENT ^a	MALE				FEMALE			
		Day 8		Day 10		Day 8		Day 10	
		Tumor wt. ^b	% inhibition						
Control		1.4 ± 0.21		2.5 ± 0.43		1.3 ± 0.19		2.0 ± 0.30	
HU: 0.2 mg	1			1.6 ± 0.14 ^d	36			1.5 ± 0.23 ^e	25
HU: 1 mg	1	0.1 ± 0.03 ^f	93	0.5 ± 0.10 ^f	80	0.2 ± 0.04 ^f	85	0.5 ± 0.04 ^f	75
HU: 5 mg	1	0.1 ± 0.04 ^f	93	0.3 ± 0.12 ^f	88	0.2 ± 0.02 ^f	85	N	
HU: 1 mg + TP: 0.5 mg	1	0.5 ± 0.01 ^f	59			0.9 ± 0.10 ^f	31	0.7 ± 0.07 ^f	65
HU: 1 mg + D: 10 mg	1	0.5 ± 0.02 ^f	59	0.9 ± 0.18 ^f	64	0.6 ± 0.06 ^f	54	1.4 ± 0.21 ^d	30
HU: 1 mg + F: 10 units	1	0.3 ± 0.04 ^f	78	0.1 ± 0.17 ^f	56	0.4 ± 0.08 ^f	69	1.3 ± 0.14 ^d	35
HU: 1 mg	7			1.7 ± 0.25 ^e	32			2.0 ± 0.47	0
HU: 1 mg	4	0.4 ± 0.04 ^f	71	1.3 ± 0.16 ^d	48	0.4 ± 0.08 ^f	69	1.4 ± 0.17 ^d	30
HU: 1 mg ^g	4	0.3 ± 0.03 ^f	78	0.9 ± 0.14 ^f	64	0.3 ± 0.06 ^f	77	1.1 ± 0.19 ^f	45

^a Day of implantation = Day 1.

^b Mean ± S.E. (gm).

^c N, no survivors; TP, testosterone propionate; D, 17 α -hydroxyprogesterone caproate (Delalutin—Squibb); F, human chorionic gonadotropin (Follutein—Squibb); HU, hydroxyurea.

^d $P < 0.05$.

^e Not significant.

^f $P < 0.01$.

^g Injection into tumor.

delayed until Day 4, the day when the tumor becomes palpable, or until Day 7, a day when rapid growth is underway. Delay of treatment until Day 4 resulted in tumor weights which were larger than those in animals where treatment was initiated on Day 1; however, significant inhibition was obtained. All treated mice sacrificed on Day 8 or 10 postimplantation had tumors

which were 30–71% smaller than those of nontreated animals sacrificed at the same time. Treatment started on Day 7 resulted in tumor weights which were smaller in the male than those of the controls, but this inhibition was not statistically significant.

Tumor inhibition may be influenced by the concentration of the antitumor agent at the site of the growth. Therefore, hy-

TABLE 2

EFFECT OF HYDROXYUREA ON H2712 GROWTH OF C3H/HeJ MICE ON DAY 11 POST-TUMOR IMPLANTATION

HYDROXYUREA TOTAL DOSE (mg)	DOSE REGIMEN	MALE		FEMALE	
		Tumor wt. (gm) ^a	% inhi- bition	Tumor wt. (gm) ^a	% inhi- bition
10	Control	2.6 ± 0.36		2.4 ± 0.32	
	1 mg for 10 days	0.7 ± 0.09 ^b	74	0.8 ± 0.10 ^b	64
	3.3 mg on Days 2, 5, 8	1.1 ± 0.16 ^b	60	1.2 ± 0.17 ^b	51
	10 mg on Day 4	1.0 ± 0.13 ^b	60	1.2 ± 0.13 ^b	50
10	10 mg on Day 6	1.6 ± 0.22 ^b	38	1.4 ± 0.20 ^b	42
25	Control	2.1 ± 0.43		2.5 ± 0.51 ^b	
	2.5 mg for 10 days	0.3 ± 0.17 ^b	86	0.5 ± 0.15 ^b	82
	3 times on Day 1	0.7 ± 0.20 ^b	65	0.7 ± 0.16 ^b	74

^a Mean ± S.E. (gm).^b *P* < 0.01.

droxyurea (1 mg/day) was injected directly into the tumor mass starting on Day 4. The results (Table 1) demonstrated that s.c. or intratumor injection produced similar degrees of tumor growth inhibition.

Dose regimen may be critical in producing efficacious treatment with a rapidly metabolizing drug. A study was therefore performed in which a total dose of 10 or 25 mg was administered under several different treatment protocols. All treatments produced significant tumor inhibition (Table 2). When the total dose (10 mg) was given intermittently on Days 2, 5, and 8, the reduction in tumor growth was slightly less but not significantly different from that produced by daily administration of the same total dose. Similarly, when the entire dose was administered in a single s.c. injection on Day 4, the result approximated that of daily or intermittent treatment. However, administering the entire dose (10 mg) on Day 6 produced tumor inhibition which was less than that obtained by the other dose schedules. Increasing the total dose of hydroxyurea to 25 mg administered in 10 daily injections resulted in tumor inhibition of 86% and 82% in the male and female mouse, respectively. When the entire 25-mg dose was administered in 3 divided doses on Day 1 tumor growth retardation resulted which was not significantly different from that produced by daily injection of this drug.

No significant sex difference in the response to this antitumor agent was observed.

Discussion

The mammary adenocarcinoma H2712 is a rapidly growing tumor readily propagated in mice and therefore lends itself to the study of antitumor compounds that are effective within a short period of time or are available in only small amounts. Several steroids which have been active in inducing remissions in human breast and uterine cancers have also been effective antitumor agents in the H2712 tumor system (10, 13).

The present investigation demonstrates that hydroxyurea is an effective agent in nontoxic doses in preventing growth of the H2712 tumor. The activity of this compound was not due to interference with the initial establishment of the neoplasm since a delay of drug administration until after growth had begun also

resulted in retarded tumor growth. It is interesting that administration of this compound every 3rd day was as efficacious as daily treatment. Even a single injection or multiple injection in a single day was as effective as daily dosage if the drug was administered prior to the onset of rapid growth of the tumor. Since it has been demonstrated that hydroxyurea interferes with DNA synthesis (5, 6, 14, 18), it can be postulated that this drug prevents tumor growth by retarding cellular proliferation. Therefore, the earlier an effective dose is administered the greater the potential for inhibition of tumor growth. If doses are employed that produce toxicity not limited to the desired target tissue, use of intermittent treatment such as described in this report can minimize such toxicity without sacrificing the antitumor effect of the drug. This reduction in toxicity may be a result of the rapid excretion of hydroxyurea (1, 3, 4) and the avoidance thereby of a sustained insult to the bone marrow. Recently, Lerner and Beckloff (9) reported a reduction in toxicity without a decrease in therapeutic effectiveness which resulted from the use of an intermittent regimen of hydroxyurea in man.

The use of steroidal and protein hormones which were previously found to be antagonistic to the growth of this mammary adenocarcinoma (10, 13) together with hydroxyurea did not result in greater antitumor activity. It would be of interest to administer hydroxyurea intermittently and alternated with one of the effective hormonal antitumor agents. Such treatment might result in enhanced tumor inhibition without increasing toxic manifestations.

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