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Fifty-five Percent Complete Remission of Mammary Carcinoma in Mice with 5-Fluorouracil and Chloroquine

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ABSTRACT

Injection of 5-fluorouracil (5-FUra) [12 mg/kg i.p. (0.1 ml) every other day] and chloroquine phosphate (CP) [10 mg/kg i.p. (0.1 ml) daily] after the 19th day postimplant brought about a 55% complete remission of mammary carcinoma (C3HBA) in 2 separate experiments. FUra used alone brought about 40% complete tumor remissions, whereas CP used alone resulted in 30% complete remissions. None of these tumors reappeared before 189 days postimplant in Experiment 1 and 120 days postimplant in Experiment 2.

The 5-FUra + CP treatment showed statistically ($P < 0.05$) smaller tumor sizes compared to control mice tumor sizes on each of 13 check days after treatment was begun. The CP-treated tumors were statistically smaller than the control tumors on 10 check days, whereas the 5-FUra-treated tumors were smaller than the control tumors on 9 check days. The 5-FUra-treated tumors were statistically smaller than the CP-treated tumors on 2 check days (23 and 33). The 5-FUra + CP-treated tumors were statistically smaller than the CP-treated tumors on 3 check days (36, 43, and 47), and the 5-FUra + CP-treated tumors were statistically smaller than the 5-FUra-treated tumors on the last 4 check days (54, 57, 61, and 64).

INTRODUCTION

A logical assumption based on the accumulated evidence of the role of cellular lysosomes in carcinogenesis and in the cancer process is that, if lysosomes could be stabilized to prevent the release of enzymes, it may be possible that certain cancers could be successfully controlled (3). Several substances have been found that are capable of stabilizing the lysosomal membranes that prevent rupture and release of the enzymes. Included among these substances are cortisone and chloroquine (4).

Therefore a recent experiment was done in which the antimalarial drug CP[2] was used to treat experimental mammary carcinoma of mice because of the reported stabilizing effects of the drug on the lysosomes of tumor cells (3). CP brought about a significant increase in survival, with a complete tumor remission occurring in 1 mouse (3). Since 5-FUra is a well-established and widely used drug in breast cancer cases, it was decided to compare the separate as well as the combined effects of 5-FUra and CP against mammary carcinoma of mice.

MATERIALS AND METHODS

A dose-response study was done with CP (Columbia Medical Co., New York, N. Y.) and 5-FUra (Roche Laboratories, Nutley, N. J.) in combination and separately. The 5-FUra was given in daily i.p. doses of 12, 24, 36, 48, 60, 72, 84, 96, and 108 mg/kg (12 mg/0.1 ml) to each of 3 mice per dosage level. All of the mice receiving daily dosages of 60 mg/kg or more died within 2 weeks. One mouse receiving 48 mg/kg died. No apparent ill effects were observed in the mice receiving 36 mg/kg or less. A similar study of CP in s.c. doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/kg resulted in only 1 death within 2 weeks at the 80-mg/kg level. The combination of 5-FUra, 36 mg/kg i.p. (0.3 ml), plus CP, 10, 20, 30, 40, and 50 mg/kg s.c., was extremely toxic; all mice died within 2 weeks.

Therefore a conservative drug regimen was used with 5-FUra given a level of 12 mg/kg i.p. (0.1 ml) every other day and CP given in 10-mg/kg amounts (0.1 ml) every day. The mice were watched carefully throughout the experiments for any possible toxic effects.

Experiment 1. Each of 80 young adult female C3H mice was given an implant s.c. (at random) in the right dorsolumbar region of 50 mg of mammary carcinoma (C3HBA) with a 12-gauge trocar. The mice were not treated until 19 days after tumor implant when the tumors had reached a measurable size [0.06 ± 0.01 cu cm (S.D.)]. Then the mice were randomly placed into 4 groups of 20. One group received daily s.c. injections of CP (10 mg/kg; 0.1 ml). Another group was given i.p. injections of 5-FUra (12 mg/kg; 0.1 ml) every other day. The third group was given daily s.c. injections of CP and every other day i.p. injections of 5-FUra. The fourth group (controls) was given daily s.c. and i.p. injections of 0.1 ml sterile water per each injection. Medication was stopped when the tumors had apparently disappeared to avoid deaths due to drug toxicity. If the tumors reappeared, drug therapy was resumed. On alternate third or fourth days from the 19th day to the 64th day, the tumors were measured with metric calipers (greatest width and length), and the volume was calculated in cu cm according to the formula, $V = \pi W^2 L/6$, for an ellipsoid or sphere (1). The mean tumor sizes for each check day were compared according to Student's t test (2 tailed). Confidence limits were set at $p = 0.05$.

Experiment 2. Experiment 2 was an exact rerun of Experiment 1. The mice of each experiment were kept separately in stainless steel cages (Hoeltge, Inc., Cincinnati, Ohio) and provided with Purina lab chow and water *ad libitum*. The weights of the mice were taken on the day of tumor implantation and again on the 50th day postimplant. Complete take was achieved with the tumor implants in each mouse in each experiment.

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[2] The abbreviations used are: CP, chloroquine phosphate; 5-FUra, 5-fluorouracil.

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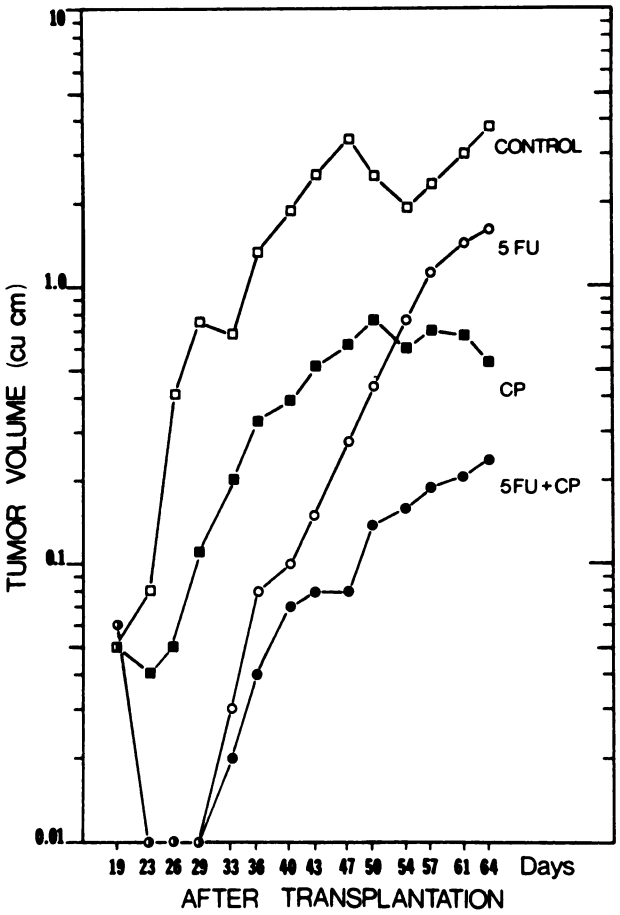

RESULTS

Experiment 1. All of the tumors of the mice receiving chemotherapy (CP, 5-FUra, or both) grew at either a greatly reduced rate, as compared to the control tumors, or completely disappeared. By 189 days postimplant, 11 mice (55%) receiving both CP and 5-FUra were alive and without any sign of tumors, 2 animals had reappearance of tumors and died, and 7 animals were still alive with the original tumor present. The 5-FUra only treatment resulted in 8 (40%) complete tumor remissions with 12 deaths, whereas the CP only treatment resulted in 6 (30%) complete tumor remissions and 14 deaths.

The 5-FUra + CP treatment showed statistically smaller tumor sizes compared to control mice tumor sizes on every check day (13 check days) after the 19th day postimplant. The 5-FUra-treated tumors were statistically smaller than the control tumors on 9 check days, whereas the CP-treated tumors were statistically smaller than the control tumors on 10 check days. In comparing the treated tumor sizes, it was found that the 5-FUra-treated tumors were statistically smaller than the CP-treated tumors on 2 check days (23 and 33). The 5-FUra + CP-treated tumors were statistically smaller than the CP-treated tumors on 3 check days (36, 43, and 47), and the 5-FUra + CP-treated tumors were statistically smaller than the 5-FUra-treated tumors on the last 4 check days (54, 57, 61, and 64) (Chart 1).

Chart 1. Logarithmic graph illustrating the mean tumor volumes (cu cm) of the 5-FUra-(*F-FU*), CP-, and 5-FUra + CP (*5-FU* × *CP*)-treated groups of mice along with the mean tumor volumes of the control mice. CP was given at a dosage level of 10 mg/kg s.c. (0.1 ml) every day, and 5-FUra was given at a dosage level of 12 mg/kg (0.1 ml) every other day.

Table 1

Days of tumor disappearances, days of tumor reappearances, and days of death of all animals that died in the treated groups

CP was given at a dosage level of 10 mg/kg s.c. (0.1 ml) every day, and 5-FUra was given at a dosage level of 12 mg/kg i.p. (0.1 ml) every other day.

	Day of tumor disappearance		Day of tumor reappearance		Day of death of all animals that died	
Drug therapy	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
5-FUra	29 35 29 35 29 35 29 35 31 38 31 42 31 33.0 ± 4.2[b]	32 32 32 35 42 56 38.2 ± 9.6	32 (86)[a] 49 (91) 60 (77) 60 (177) 38 (89) 47.8 ± 12.7	None reappeared	61 78 63 84 67 86 67 89 70 91 77 177 84.2 ± 12.9	60 78 63 79 63 83 64 87 65 100 68 110 76 114 79.3 ± 17.8
CP	29 35 31 42 35 45 35 49 38.0 ± 7.0	42 49 60 50.3 ± 9.1	84 (161) 66 (78) 75.0 ± 12.7	None reappeared	40 78 47 86 53 115 61 147 62 161 67 175 70 188 96.4 ± 50.8	49 65 52 66 53 71 55 80 64 87 64 92 64 104 65 116 74.3 ± 21.5
5-FUra + CP	29 42 31 42 31 42 35 45 38 49 38 49 42 40.0 ± 6.8	32 42 32 46 39 46 39 53 39 60 42 42.7 ± 8.4	52 (115) 60 (133) 56.0 ± 5.7	None reappeared	115 133 124.0 ± 9.0	No deaths before 120 days

[a] Numbers in parentheses, day of death.
[b] Mean ± S.D.

Complete tumor remission occurred on Day 40 (±6.8) in 14 of the 5-FUra + CP-treated mice. Three mice had tumors reappear on Day 55 (±4.0), and all 3 mice died (113.7 ± 20.0 days). In the 5-FUra-treated mice, complete remission occurred on Day 33 (±4.2) in 13 mice. Five of these mice had tumors reappear on Day 48 (±12.7), and all 5 mice died (104.0 ± 36.5 days). The CP treatment resulted in 8 tumor remissions by Day 38 (±7.0), with recurrences occurring in 2 mice on Day 75 (±12.7); both mice died (Days 78 and 161) (Table 1). All of the control mice died (64.9 ± 18.1 days).

The 50-day weight gains were: 5-FUra + CP mice, 16.9 ± 0.6 g to 22.8 ± 1.5 g; 5-FUra only mice, 16.9 ± 0.6 g to 23.3 ± 2.5 g; CP only mice, 16.9 ± 1.2 g to 21.6 ± 2.6 g; and control mice, 16.9 ± 0.6 g to 25.7 ± 3.2 g.

Experiment 2. The results of this repeat experiment (based on the 120th day postimplant) are almost an exact copy of the first experiment. Eleven mice (55%) receiving both 5-FUra and CP were completely without apparent tumors, and all 20 mice lived beyond 120 days. The 5-FUra only treatment resulted in 6 (30%) complete tumor remissions; the mean day of death of the others was 79.3 ± 17.8 days (Table 1). The CP only treatment resulted in 3 (15%) complete tumor remissions; the mean day of death was 74.3 ± 21.5 for the others (Table 1). All of the control mice died at 68.5 ± 19.2 days.

DISCUSSION

It is of interest that some tumors reappeared after having apparently disappeared in Experiment 1, whereas no tumors reappeared after the initial disappearance in Experiment 2. Why this occurred is not known.

On the basis of our experiment with CP treatment (3), it is clear that the combined 5-FUra + CP treatment is superior to CP treatment alone. Also, it is clear that the combined 5-FUra + CP treatment is more effective than 5-FUra alone treatment, as CP appears to have an additive effect to 5-FUra.

Recently, it has been pointed out that early systemic treatment of a breast cancer patient with appropriate chemotherapy has improved survival (2). Abundant evidence exists from work with experimental tumors that early chemotherapy is more successful in attaining cure than treatment of the gross tumor. This improved result is dependent in part upon relatively more circulation and thus drug delivery to each tumor cell, a higher proportion of cells in the active cell cycle, and greater vulnerability to interference with the biochemical processes. Furthermore, a healthier and more resilient host, without compromise of immune competence by tumor effects or by cachexia is better able to withstand the drug effects on normal tissues and to participate in tumor destruction (2). The present study may lend support to the view that chemotherapy, if begun at the time of tumor detection, is more effective and may be continued for a longer period of time.

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