Inhibition by 5-Fluorouracil of the Early Stages of Chemical Carcinogenesis in Mouse Skin (34054)

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Prophylactic inhibition of chemical carcinogenesis could be of value in elucidation of pathological mechanisms which result in carcinogenesis as well as for applied purposes (1). As a means of investigating this type of inhibition, experiments have been carried out in which the inhibitory effects of compounds are determined when administered in the interval between carcinogen application and the first appearance of neoplastic lesions of the skin of the mouse. In the experimental system used in the present investigation, four large doses of 7,-12-dimethylbenz(*a*)anthracene (DMBA) are applied to the skin of a mouse. An interval of approximately 4 weeks exists between the last application of the carcinogen and the first gross evidence of neoplasia. During this interval, compounds can be administered in order to test their inhibitory effects on the early stages of carcinogenesis. Topical administration has been used in order to obtain high local concentrations of compounds with minimal systemic levels. In the present work, two compounds employed clinically in cancer chemotherapy have been tested. The compounds are 5-fluorouracil (5-FU) and cytoxan. Both compounds are usually administered systemically in cancer chemotherapy, but they also have been applied topically for the therapy of keratoses and small malignant tumors of the skin (2–4).

Materials and Methods. In all of the experiments, female Ha/ICR mice obtained from Millerton Farms, New Jersey, were employed. Mice 2 months of age were used in the first two experiments. An area of approximately 4 × 1.5 cm on the back was shaved and the animals observed for 4 days for hair regrowth. Only animals not showing hair regrowth were used. In the first experiment, 200 μg of DMBA contained in 4 drops of acetone with 1% mineral oil was dropped on the shaved area four times in a period of 10 days. There were 3-day intervals between each of the initial three doses and a 4-day interval between the third and fourth dose.

One week after the last DMBA administration, the animals were placed into two groups of 26 mice each of approximately equal average body weights. One group of mice was then treated with 1% 5-FU in propylene glycol once daily 6 days a week for 2 weeks. The other was treated with propylene glycol for the same time. Both solutions were applied to the skin of the back by means of a No. 7 camel-hair brush. Approximately 0.6 cc of solution was applied per mouse as determined by the volume of solution employed. Subsequent to each application, the mouse was immediately placed in a narrow wire tube for 1 hour in order to prevent licking. The tubes were constructed from galvanized wire mesh and measured 3½ in. in length and 1 in. in diameter. One end of the tube was permanently closed and the other end had a door. The number of tumors and their size and also the body weights of the mice were recorded at 2-week intervals.

The second experiment was started after completion of the initial experiment. Similar conditions were employed. However, the dosage of DMBA was reduced to 150 μg per application and the drugs were administered 2 weeks after the application of DMBA. Six groups, each containing 31 mice, were studied. These received application of 1% 5-FU, 0.2% 5-FU, 1% cytoxan, 0.2% cytoxan, vehicle (propylene glycol), or nothing.

The third experiment was of a different nature from the first two. In this investiga-

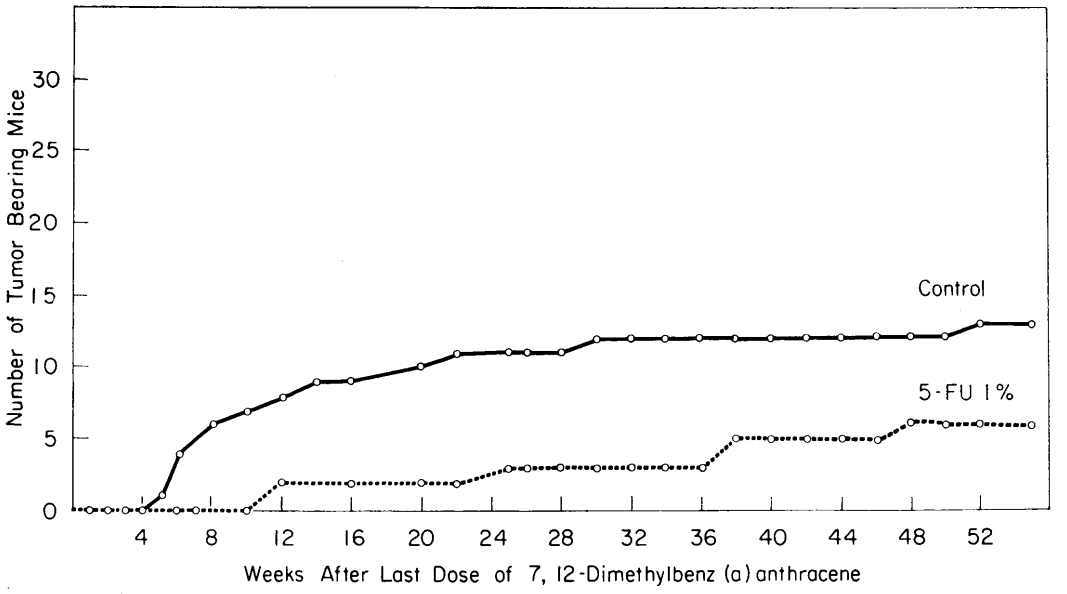

FIG. 1. Effect of 5-FU on DMBA-induced epidermal carcinogenesis of mouse skin.

tion, the capacity of 5-FU to produce regressions of skin tumors was studied under similar conditions to those employed in the first two experiments. The skin tumors were produced by four applications of 150 μg DMBA each as described above except that the mice were 1 month of age at the time they received the initial dose of DMBA. This was done so that the age of the animals at the time of the 5-FU treatment would be comparable in all experiments. Two months after the DMBA applications, animals with tumors 2×2–6×7 mm were randomized into four groups, each containing nine mice. Each group had an approximately equal number of tumors of similar sizes. Groups were also arranged so that the average body weights of the animals were within 1 g. The same techniques for brushing and caging were used as in Expts. 1 and 2. One group of mice received 1% 5-FU; the second 0.2% 5-FU; the third, vehicle (propylene glycol); and the fourth received nothing. Twelve applications were administered in a period of 2 weeks.

Results. The results of Expt. 1 are depicted in Fig. 1 and also included in Table I. It will

TABLE I. Inhibition of Carcinogenesis in Mouse Skin.

			25 Weeks after the last dose of DMBA					
Expt. no.	Experimental group	Control weight (g)	No. of survivors	Weight (g)	No. of mice with tumors	% Mice with tumors[a]	No. tumors	Tumors/ mouse[a]
1	Solvent control	23.4	18	29.8	11	42.3	17	0.65
	5-FU 1%	23.4	24	31.8	3	11.5	3	0.12
2	Solvent control	29.5	26	34.1	8	25.8	9	0.29
	5-FU 1%	29.8	29	34.3	1	3.2	1	0.03
	5-FU 0.2%	30.2	28	35.2	7	22.6	10	0.32
	Cytoxan 1%	30.2	26	35.7	8	25.8	9	0.29
	Cytoxan 0.2%	29.7	27	33.8	8	25.8	10	0.32
	Absolute control	30.0	22	34.7	13	41.9	16	0.52

[a] Twenty-six mice per group in Expt. 1 and 31 mice per group in Expt. 2.

TABLE II. Regression of Epidermal Tumors of Mouse Skin.

Experimental group	No. of mice	Body weight (g)			No. of tumors	No. of tumor regressions
		Control	Completion of 5-FU	End of expt.		
Solvent control	9	27.0	28.9	28.7	17	0
5-FU 1%	9	27.3	25.2	29.3	13	11
5-FU 0.2%	9	27.3	29.4	30.3	13	0
Absolute control	9	26.8	28.6	29.4	12	1

be noted that the latent period before the appearance of tumors in the group of mice receiving 1% 5-FU was considerably greater than that of the controls and the tumor incidence was considerably less. The results obtained in Expt. 2 are also presented in Table I. As in the initial experiment, 1% 5-FU had a significant inhibitory effect. In contrast, 0.2% 5-FU and both dose levels of cytoxan did not result in an inhibitory effect as compared to the solvent control group. It will be noted that the animals that were kept as absolute controls, that is to say, without any treatment after receiving the carcinogen applications, had a higher tumor incidence than the solvent controls that received propylene glycol. This effect is under further study.

The results obtained in Expt. 3 are shown in Table II. Administration of 1% 5-FU caused regression of 11 of the 13 tumors treated. The animals receiving 0.2% 5-FU did not show tumor regression.

Discussion. The present study has clearly demonstrated that it is possible to inhibit chemical carcinogenesis by administration of 5-FU shortly after cessation of carcinogen application and prior to gross evidence of neoplasia. *A priori*, it might be anticipated that the early states of carcinogenesis would be relatively readily reversible. Some encouragement for this possibility is found in the work of Crabtree in which high concentrations of bromobenzene or maleic anhydride were found to inhibit epidermal carcinogenesis in the mouse under an experimental system similar to the one employed in the present study (5, 6). In addition, more recent investigations have shown that actinomycin D, dropped on to mouse skin shortly after DMBA applications, will reduce the number of papillomas that occur (7, 8). However, in the present work, it appears that the dose required to obtain inhibition of the early stages of carcinogenesis is of the same order of magnitude as that which is necessary to produce tumor regression. In other words, with 5-FU, the early stages of carcinogenesis are not particularly sensitive to reversal, and in the case of cytoxan do not respond at all. Nevertheless, it is quite possible that with an appropriate agent, conceivably one quite different from those used in conventional cancer chemotherapy, a highly selective effect might be obtained. It would seem worthwhile to pursue this objective.

Summary. 5-FU was found to inhibit the early stages of DMBA-induced epidermal carcinogenesis in the mouse. The dose which must be employed to obtain this effect is of the same order of magnitude as that required for bringing about regression of established tumors of the mouse skin.

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Received March 24, 1969. P.S.E.B.M., 1969, Vol. 131.