Effect of Drugs on Cell and Virus Growth in Friend Leukemia and a Tumor Variant.* (30137)

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The leukemia induced by Friend virus because of its short latent period is well suited for use as a test system for evaluating anticancer agents. Using the parameters of mortality, splenic size and splenic uptake of Fe^{59}, the effects of a large number of compounds on Friend leukemia have been studied(1,2). In any virus-induced leukemia there are two related, but separate processes, namely cellular proliferation and virus replication. Experiments were designed to determine whether agents previously shown to be effective in controlling the cellular proliferation in this leukemia influenced virus replication in the tissues. This was determined using both (a) a transplantable tumor variant and (b) cell-free virus preparations. The cellular response was expressed in terms of spleen and tumor weight; virus content of these tissues was determined by bioassay.

Materials and methods. The preparation of Friend virus and the transplantable reticulum cell sarcoma variant have been described (3,4). Urethane, triethylene melamine (TEM), 6 mercaptopurine (6MP) and mitomycin C were obtained by courtesy of the Cancer Chemotherapy National Service Center, Washington, D. C. Estradiol benzoate was purchased from Nutritional Biochemicals Inc., Cleveland, Ohio. Estradiol and 6MP were taken up in carboxymethyl cellulose (CMC), mitomycin C in phosphate buffer pH 7.0 and the other compounds in saline. All solutions were prepared daily and injected intraperitoneally in 0.5 ml of the vehicle, except for estradiol which was injected in 0.2 ml of CMC subcutaneously. The dosages used are given in Tables I and II. Young female Balb/c mice originating from the Cancer Research Genetics Laboratory, Berkeley, Calif., and young random bred female Swiss mice from the Hooper Foundation, San Francisco, Calif., were used.

A group of mice bearing the eleventh passage of the reticulum cell sarcoma variant was killed, the tumors removed aseptically and pooled. Necrotic tissue was discarded and the viable portions of the tumors were

TABLE I. Effects of Estradiol and Mitomycin C on Mice Infected with Friend Virus.

Virus dose ($-\log_{10} ID_{50}$)	Drug	Daily dose (mg/kg)	Avg spleen wt (g ± SD)	No. with FD histologically/ No. inoculated	Virus titer of spleen per ml ($-\log_{10} ID_{50}$)
3.4	None		1.94 ± .88	39/39	5.2
3.4	Estradiol	25.0	.39 ± .10	25/39	4.4
0	None		.15 ± .04	0/10	0
0	Estradiol	25.0	.12 ± .02	0/10	0
2.7	None		1.80 ± .75	25/25	3.4
2.7	Mitomycin C	1.0	.11 ± .05	14/27	2.6
0	None		.14 ± .05	0/18	0
0	Mitomycin C	1.0	.09 ± .03	0/30	0

SD = standard deviation. FD = Friend disease.

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TABLE II. Effect of Drugs on Mice Bearing a Transplantable Reticulum Cell Sarcoma Variant of Friend Leukemia.

Drug	Daily dose (mg/kg)	Control animals* Avg spleen wt (g ± SD)	Results: Spleen Avg wt (g ± SD)	Spleen No. with FD histologically/ No. inoculated	Spleen Virus titer per ml ($-\log_{10} ID_{50}$)	Tumor No. with tumors/ No. inoc	Tumor Avg wt (g ± SD)	Tumor Virus titer per ml ($-\log_{10} ID_{50}$)
Saline		.14 ± .02	.43 ± .40	10/21	4.6	21/21	.78 ± .74	4.3
CMC		.13 ± .02	.28 ± .16	10/22	4.6	21/22	1.05 ± 1.02	3.7
Urethane	500	.09 ± .02	.18 ± .05	9/21	4.3	21/21	.32 ± .22	4.1
Mitomycin C	2	.06 ± .02	.05 ± .01	0/8	1.0	5/8	.02 ± .01	Not tested
Estradiol	40	.08 ± .02	.19 ± .11	13/27	4.1	27/27	.67 ± .35	4.0
6MP	30	.12 ± .01	.29 ± .13	13/27	4.1	25/27	.21 ± .18	3.9
TEM	.5	.06 ± .01	.14 ± .07	14/27	4.2	23/27	.18 ± .16	4.3

SD = standard deviation. FD = Friend disease. * 10 mice in each group.

cut into 2 mm pieces and suspended in phosphate buffered saline containing antibiotics. Balb/c mice were anesthetized with intraperitoneal nembutal and a single piece of tumor implanted into the right axilla through a surgical incision which was closed with catgut. The animals were randomized in groups of 30 and starting on the first post-operative day were treated according to the schedules in Table II. Control groups of 10 mice were subjected to the same operative procedure and treatment, except that no tumor was implanted. Twenty-one days postoperatively the animals were sacrificed. Tumors and spleens were removed aseptically, weighed, and after samples had been taken for histology they were quick frozen and stored overnight at —70°C. Control animals were sacrificed at the same time, their spleens weighed and examined histologically. Except the tumors from animals treated with mitomycin, which were too small to process, the frozen tissues from each group were thawed, homogenized at 2 to 4°C in sucrose-phosphate buffer and decimally diluted from 10^{-1} through 10^{-5} in buffer. Each dilution was inoculated into 10 Swiss mice. Thirty-five days later the mice were killed and their spleens weighed. Those larger than 0.5 were considered positive for Friend disease, the remainder were examined histologically. The ID_{50} was calculated from the total of those showing macroscopic and microscopic disease.

In another series of experiments, groups of Balb/c mice were injected intraperitoneally with Friend virus and starting on the next day treated with either estradiol or mitomycin C in the dosage given in Table I. Control animals received Friend virus, estradiol, mitomycin C and no treatment respectively. Twenty-one days after inoculation the mice were sacrificed, their spleens removed aseptically, processed and titrated as described above.

Results. These are detailed in Tables I and II. Both estradiol and mitomycin C significantly reduced the incidence and rate of progression of Friend disease as judged by the size and the histologic appearance of the spleens of mice receiving virus extracts,

There was, however, no significant difference in the virus titer of the spleens in control and test animals.

Among animals receiving the transplantable tumor, mitomycin C, TEM, 6MP and urethane, all significantly inhibited tumor growth, although the incidence of takes in test and control animals surviving to the end of the experiment was about the same. Estradiol had little effect on rate of tumor growth. The titer of virus in the tumors and spleens showed no significant differences between test and control animals. Mitomycin C proved toxic in the dosage given and the number of survivors was small; this accounted for our failure to titrate the tumors, and may also account for the low virus titer in the spleen. The spleens of tumor-bearing animals were smaller than those encountered in animals receiving cell-free virus preparations. However, the spleens in the former always yielded slightly more virus than the tumors, findings in agreement with those reported previously(5).

Discussion and summary. The results show that neither estradiol nor mitomycin C influence the replication of Friend virus as judged by virus titer in the spleen, although both inhibit development of the leukemia following inoculation of cell-free virus(1,2).

The results also show that mitomycin C, TEM, 6MP and urethane inhibited the growth of cellular implants of a transplantable reticulum cell sarcoma variant of Friend leukemia, although none of these substances affected the concentration of virus in either the spleen or tumor. Estradiol occupied an anomalous position in that it inhibited the development of splenomegaly in animals receiving both cell-free virus and tumor transplants, but did not inhibit the growth of the subcutaneous tumor.

While these drugs influence the response to Friend virus, it seems likely that their withdrawal, even after complete suppression of the original cellular response, would be followed by recrudescence of leukemia from new foci of neoplasia induced by the large amount of virus in the tissues. Evidence to support this concept has been reported for Maloney virus by Glynn *et al*(6). The fallacy of using only one parameter in assessing the results of chemotherapy is highlighted. In the case of virus-induced neoplasms, treatment should ideally inhibit the growth of both the neoplastic cells and the virus.

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Effect of Hypothalamic Extracts on Release of Growth Hormone *in vitro*.* (30138)

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Although the impairment of growth in rats and dogs with hypothalamic lesions and a significant fall in pituitary growth hormone content of lesioned rats have been demonstrated(1-4), direct evidence of hypothalamic regulation of growth hormone secretion has been lacking.

Recently Franz *et al*(5) claimed the pres-

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