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Inhibition of Growth of Mouse Mammary Carcinoma by Streptovaricin[1,2] (38029)

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During the past two decades thousands of chemicals have been tested for their antitumor effect in animals and cell cultures. Although remarkably few have shown promise for application to humans, the main advances in cancer therapy during this period have been in this area. Almost all of the antitumor drugs in general use, however, are highly toxic and often reduce the patients' natural defenses. We describe here an antibiotic which is both nontoxic, nonimmunosuppressive and which exerts a marked inhibitory effect on tumor growth.

Streptovaricin (The Upjohn Co., Kalamazoo, MI) is a complex of ansa macrolide antibiotics isolated from *streptomyces spectabilis*. Borden *et al.* (1) reported that oral administration of streptovaricin complex reduced Rauscher leukemia virus (RLV) induced splenomegaly in Balb/c mice by 50% when treatment was started 24 hr prior to infection and by 20% when the therapy was delayed until 8 days after RLV inoculation when the spleen enlargement had already progressed considerably. Carter *et al.*, from the same laboratory (2) observed that streptovaricin complex and streptovaricin D inhibited the formation of foci in Balb/3T3 cell cultures infected with Moloney sarcoma virus. In an earlier study, Brockman *et al.*, again from the same institute (3) found that streptovaricin complex as well as streptovaricins A and C inhibited the RNA dependent DNA polymerase (RT) activity of Moloney leukemia virus. Gurgo *et al.* (4), however, found no significant inhibition by streptovaricins of RT activity of Moloney sarcoma virus.

We undertook to extend these studies to murine mammary adenocarcinomas in RIII mice. These tumors have a *B* type virus etiology (5, 6). The mammary tumor virus (MTV) is an oncorna virus both morphologically and immunologically distinct from the murine leukemia–sarcoma virus complex (7). Like the leukemia viruses, MTV has RT activity (8). Murine mammary adenocarcinoma has served as an experimental model for breast cancer research in women (9).

Materials and Methods. Mice. MTV-positive RIII mice were used to study the therapeutic effect of streptovaricin. RIII mice have a mammary tumor incidence of 96%. Mice with small palpable mammary tumors were paired as to tumor size. One of each pair was placed in the experimental group, the second was used as a control.

MTV-negative C57BL mice with a mammary tumor incidence of 0% were used in studies on a possible prophylactic effect of streptovaricin on mammary tumorigenesis. C57BL mice are highly susceptible to infection with MTV derived from RIII milk, and when infected have a mammary tumor incidence of 85%.

Virus. The virus involved in both groups of experiments was derived from RIII milk. In the therapeutic experiments the virus

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was introduced into the mice with their mother's milk; in the prophylactic group weanling mice were inoculated intraperitoneally with a fully infective dose of MTV (0.1 ml skim milk diluted 1:1 × $10^{3.5}$ with phosphate buffered saline containing 0.1% bovine serum albumin).

Antibiotic treatment: Streptovaricin complex (Lot 11560-5) was administered orally to the experimental mice in powdered Purina lab chow. The chow contained 0.5% of the antibiotic, 10% grated cheese and 10% sucrose. Control mice were fed the same diet minus the antibiotic. Each mouse ate approximately 5 g of diet per day which amounts to a daily oral intake of 25 mg of streptovaricin complex. Borden *et al.* (1) reported that mice similarly fed a diet containing 0.5% streptovaricin complex attained a drug level of 1–3 μg/ml in their serum.

Results. Two experiments were carried out to study the therapeutic effect of streptovaricin complex on established mammary tumors. In these experiments the tumors were measured at weekly intervals. Tumor growth or regression, the occurrence of new primary tumors, and the complete disappearance of palpable tumors during the experimental period were noted. The average tumor radius for each tumor was used as a measure of tumor size. Tumor measurements were made on the living mouse in two dimensions with a pair of dividers and the third dimension was considered to be twice the distance the tumor protruded from the surface of the body. The mean radius was obtained by dividing the sum of the three diameters by six.

In the first experiment the treatment was discontinued after five weeks and both experimental and control mice were fed a standard diet of pelleted Purina lab chow thereafter. In the second experiment the treatment was continued until the mice died. The mice in the first group included a number of pregnant mice while in the second group pregnant mice were excluded. It has been reported that pregnancy stimulates mammary tumorigenesis in mice (10).

The results of the two experiments are shown in Figs. 1 and 2. Figure 1 indicates that streptovaricin reduced the growth rate of mammary tumors by 51% over a 5 week period (tumor growth increased rapidly after treatment was discontinued). Figure 2 shows a reduction of 87% over

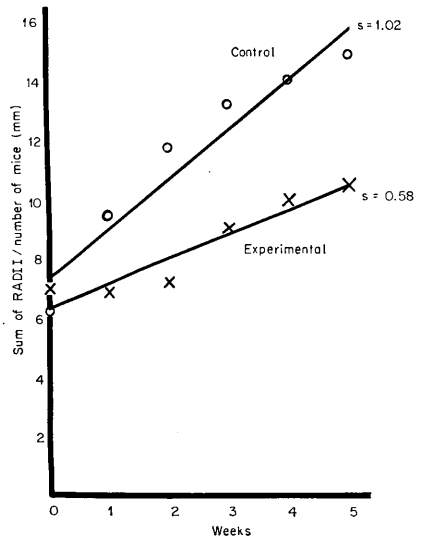

FIG. 1. Experiment 1. Effect of streptovaricin complex on the growth rate of mammary tumors in RIII mice. Control group: 7 mice; experimental group: 8 mice. s = standard error of estimate.

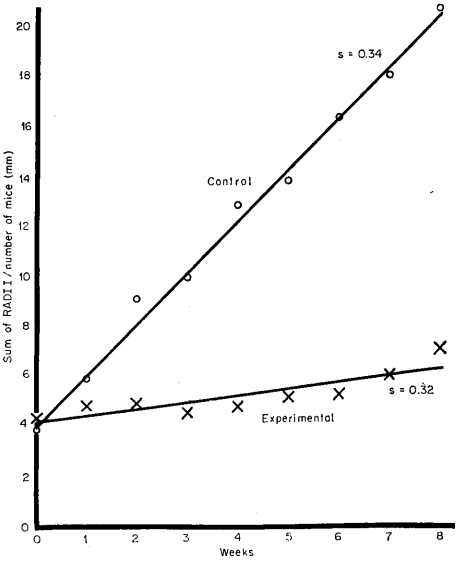

FIG. 2. Experiment 2. Effect of streptovaricin complex on the growth rate of mammary tumors in RIII mice. Control and experimental groups: 9 mice each. s = standard error of estimate.

an 8 week period. In this experiment 9 new primary tumors arose in the control group and 2 in the experimental mice. Three out of the nine test mice became tumor free compared to 0 out of 9 of the control mice. A statistical analysis of the data presented in Figs. 1 and 2 showed that the probability that the differences in tumor growth rates in the experimental and control groups could be attributed to chance is vanishingly small, with $P \ll 0.001$ by Student's test.

The cumulative death rates of the mice in the two experiments are shown in Figs. 3 and 4. Streptovaricin therapy prolonged the life span of the mice in both experiments. In experiment 1, where treatment ended after 5 weeks, 2 out of 8 treated mice (25%) survived for at least 15 weeks while all control mice (7/7) were dead within 12 weeks. When treatment was continued to the end of the period, 4 out of the 9 treated mice (44%) survived for 15 weeks against 1 of the 9 control mice (11%).

Tumor free adult female RIII mice were pair-fed the streptovaricin and control diets over a 6 week period. The mice fed the streptovaricin diet had a mean weight gain of 0.2 grams per mouse, while those fed the control diet showed a mean weight loss of 0.5 g per mouse. This difference is insignificant and indicates no deleterious effect of the streptovaricin.

In order to ascertain whether streptovaricin has any prophylactic effect on MTV infection we carried out two experiments. In the 1st experiment 50 mice were fed the experimental and 40 the control diet. The mice were challenged with a fully infective dose of MTV one day after initiation of the experiment. From the 8th day all mice were placed on our standard diet. In the 2nd experiment 20 mice were fed the experimental and 20 the control diet. In this experiment the mice were challenged on the 5th day after the start of the experiment and returned to our standard diet on the 11th day.

The mice were subsequently tested at the 3rd lactation for the presence of MTV in their milks by the procedure of Charney

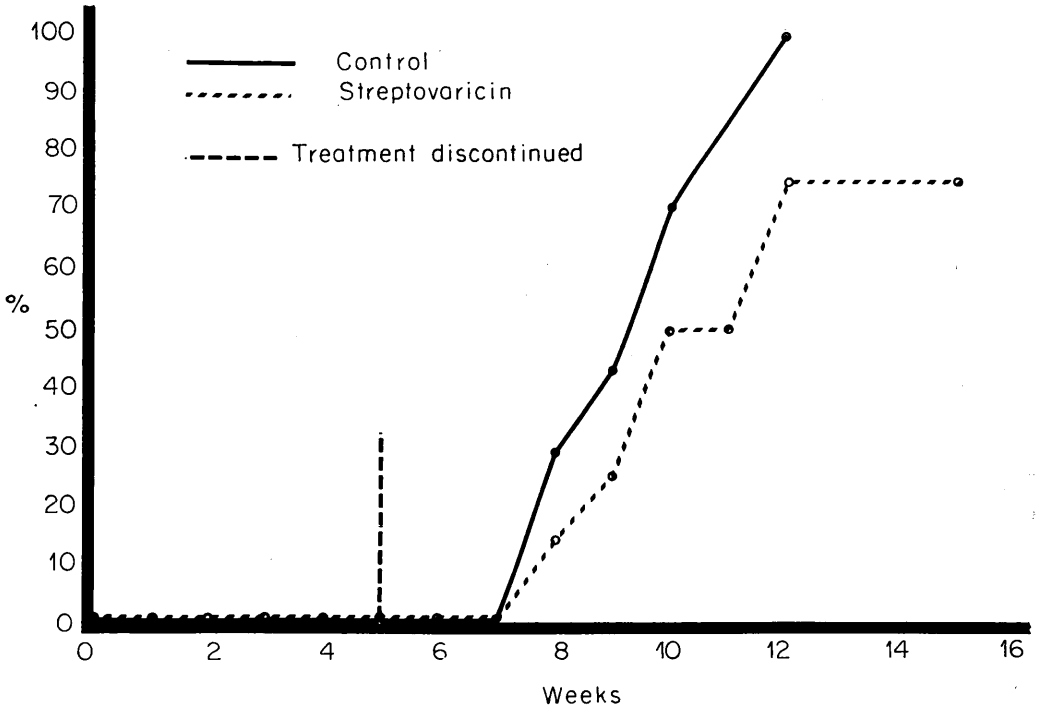

FIG. 3. Experiment 1. Effect of streptovaricin complex on the survival of tumor bearing RIII mice.

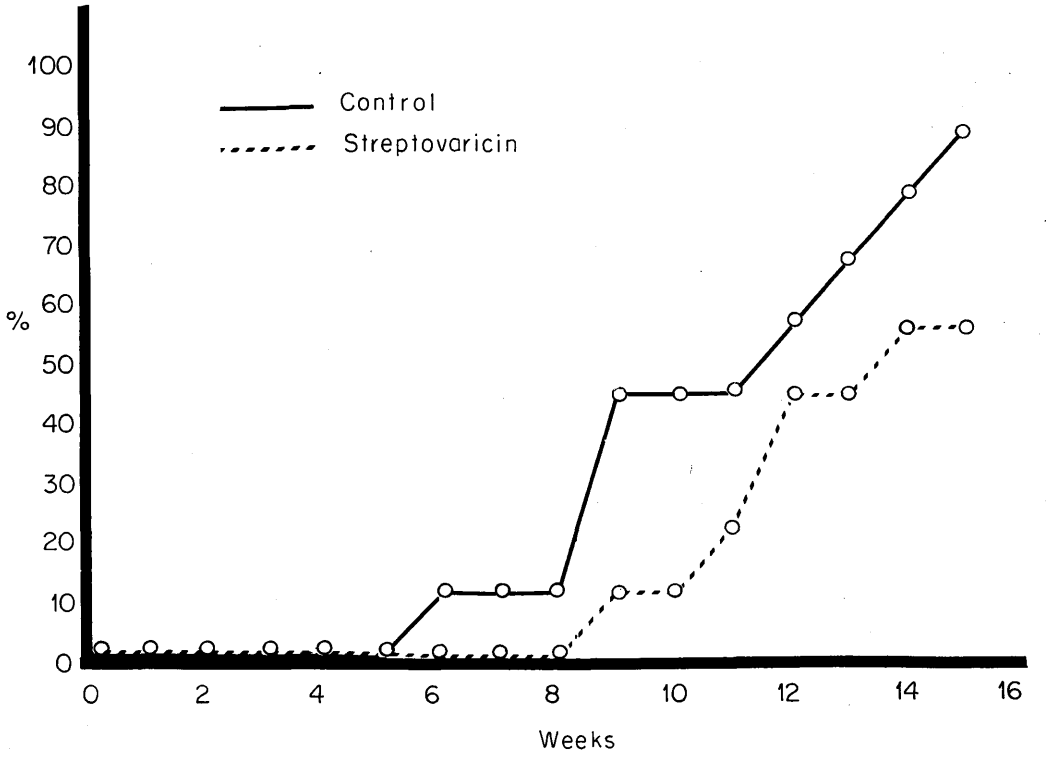

FIG. 4. Experiment 2. Effect of streptovaricin complex on the survival of tumor bearing RIII mice.

et al. (5). The results of these experiments are shown in Table I. No significant reduction in infectivity can be shown in either experiment, indicating that under the conditions of these two experiments streptovaricin had no prophylactic effect on MTV infection of mice.

Weight determinations were not made in this experiment; the dosages selected were those previously determined to be nontoxic and which did not reduce weight gain in weanling mice (1).

TABLE I. Effect of Streptovaricin Complex on MTV Infection of C57BL (MTV Negative) Mice.[a]

Treatment	Experiment No.	MTV Antigen mice positive/ mice tested (%)
Streptovaricin	1	30/39 77%
Control	1	30/37 81%
Streptovaricin	2	13/20 65%
Control	2	16/19 86%

Discussion. Streptovaricins have several advantages over some of the other RT inhibitors; (a) the drug is readily available in quantities large enough for prolonged *in vivo* testing; (b) streptovaricin inhibits leukemia-virus-induced splenomegaly; (c) administration of the antibiotic has been shown to have no deleterious effect upon the immunocompetence or the ability to produce interferon in mice (1).

The reduction in the rate of mammary tumor growth in mice treated with streptovaricin and the decrease in the number of new tumors arising in them and the complete disappearance of tumors in some raise the hope that this antibiotic or some derivative of it may someday be useful in treating breast cancer in women.

Summary. The growth of preexisting mammary tumors and the formation of additional tumors in MTV-positive RIII mice were significantly inhibited by streptovaricin complex. Mice bearing small tumors were given streptovaricin orally in two

experiments and the rate of growth of their tumors was compared with that of matched controls. In the 1st experiment treatment was discontinued after 5 weeks while in the 2nd the animals were treated until they died. The growth rates in the two treated groups were 49% and 13% of the rates in the respective control groups. Measurements were made for 5 weeks in the 1st experiment and for 8 weeks in the second. Fewer new tumors arose in the experimental mice than in the controls. More tumors regressed in the treated mice than in the controls. Short term prophylactic treatment of MTV-negative C57BL mice with streptovaricin complex failed to protect the mice significantly from infection with MTV.

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