The Effect of Actinomycin D on Experimental Ascitic Tumors in the Mouse*

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The actinomycins, a family of antibiotics unique in possessing a peptide grouping attached to a chromophore, have been shown by several investigators to possess varying degrees of antitumor activity. Hackmann (3), using in vitro technics, and Field (2), Farber (1), and Reilly (6), using experimental solid tumors in the mouse, have reported on the antineoplastic activities of the actinomycins. Similar studies have not been reported with ascitic tumors, which now are being used widely as an experimental tool in cancer research and represent a sensitive method for the detection of antitumor activity. The investigation here reported was initiated to elucidate the effectiveness of actinomycins in tests with ascitic tumors and in particular to assess the potential value of actinomycin D (5) as an antitumor agent.

MATERIALS AND METHODS

Two ascitic tumors were employed, the Crocker Sarcoma 180 and the Gardner lymphosarcoma (6C3HED). The sarcoma was obtained from Dr. C. Chester Stock of the Sloan Kettering Institute for Cancer Research and was transplanted weekly in albino male mice obtained from Harpaul Farms, New Brunswick, N.J. The other tumor came from the laboratories of Dr. Theodore Hauschka, formerly of the Lankenau Hospital, Institute for Cancer Research, and also was transplanted at weekly intervals in inbred C3H male mice supplied by the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The actinomycin D was prepared at the Institute of Microbiology and was recrystallized to constant physical properties. Solutions of actinomycin for treatment were made in sterile physiological saline in such a manner that the desired concentration could be injected intraperitoneally in 0.5-ml amounts. Control mice, given injections of saline only, were maintained in all experiments.

In studies with Sarcoma 180, ten mice per group were given intraperitoneal inoculations of 0.1 ml fresh ascitic fluid containing approximately 110 million cells/ml, from donors that had been implanted with tumor cells 7 days previously. A similar method was employed with the lymphosarcoma except that the ascitic fluid usually contained 300 million cells/ml. Treatment was initiated the day after transplantation of the lymphosarcoma and 3 days after transplantation of Sarcoma 180. Ascitic fluid was removed aseptically daily for a period of 1 week and at frequent intervals thereafter, according to the method of Hata et al. (4). The total cell count of the various samples was calculated, and the percentage of each cell type was determined by examination of Giemsa-stained smears.

Several criteria were used as indices of activity: changes in body weight, total number of cells in the ascitic fluid, gross morphological changes in tumor cells, and extension of survival time of treated mice over that of saline controls.

RESULTS

Table 1 gives a summary of the results obtained with actinomycin D against Sarcoma 180 in a typical trial in which increases in weight gain due to ascites and average survival time were the criteria employed to evaluate activity. Chart 1 indicates the results obtained with Gardner lymphosarcoma and actinomycin D. Cytological studies of Giemsa-stained preparations of ascitic fluid from mice with lymphosarcoma revealed a marked reduction in the percentage of tumor cells with low mitotic activity after treatment with actinomycin D. In both of these tests a slight increase in survival time of the treated animals was observed. It can be noted that at 8 months, two of the five mice in the lymphosarcoma group were still alive. One of these animals, with a large metastatic solid tumor in the cervical region, was sacrificed on the...
77th day of the experiment. The tumor isolated from this animal was transformed to the ascitic form in one intraperitoneal passage, and it is being studied further because several atypical characteristics were found to be associated with this apparently modified tumor.

Since it was felt that repeated withdrawals of ascitic fluid could have influenced the survival time of the mice, the experiment with the lymphosarcoma was repeated, with twenty mice per group instead of the five used previously and without the studies on the ascitic fluid. The results are presented in Table 2. Four mice died early with mycin D selectively affected tumor cells, an aseptic peritonitis was produced in normal albino mice by intraperitoneal injection of a sterile saline suspension of siliceous earth. The peritonitis was nonfatal, and the ascites that developed was at first rapidly progressive and disappeared by the 8th day (Chart 2). The sterile ascites failed to respond to actinomycin therapy regardless of the variation in dosage regimen. It disappeared spontaneously in both treated and untreated animals, and actinomycin treatment did not alter significantly the numbers of leukocytes in the ascitic fluid as compared with the controls.

### Table 1

<table>
<thead>
<tr>
<th>TUMOR-BEARING MICE</th>
<th>MEDIAN SURVIVAL TIME (DAYS)</th>
<th>NORMAL MICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMOUNT OF ACTINOMYCIN D (µg)</td>
<td>Initial weight (gm.)</td>
<td>Change in weight after days 6</td>
</tr>
<tr>
<td>TREATMENT SCHEDULE*</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>None</td>
<td>22.4</td>
<td>+3.5</td>
</tr>
<tr>
<td>Saline control</td>
<td>22.0</td>
<td>+3.2</td>
</tr>
<tr>
<td>1</td>
<td>Daily for 4 days</td>
<td>22.4</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>22.4</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>22.4</td>
</tr>
<tr>
<td>3</td>
<td>Daily for 5 days</td>
<td>21.0</td>
</tr>
<tr>
<td>5</td>
<td>1st and 4th day</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Treatment was started on the third day after tumor cell implantation.

### Discussion

Under the conditions of the present test with the Sarcoma 180 ascitic tumor, changes in body weight could be used as a criterion of efficacy only in cases where the potential antineoplastic agent itself did not cause changes in body weight. Examination of Table 1 indicates that actinomycin D reduced the weight gain of normal animals as well as of those with ascitic tumors. Therefore, extension of survival time observed in animals treated with actinomycin was relied upon for evaluation of the antitumor activity. As could be expected, owing to the toxic nature of this drug, the correct dosage regimen was quite critical. In the present study, 1.5 µg/mouse, once daily for 4 successive days (a total of 6.0 µg.), appeared best, being superior to 6 µg. in three successive doses or in two doses with 3-day intervals.

Since the Gardner lymphosarcoma grows only in inbred mice, it was hoped that this ascitic tumor would lend itself better to chemotherapeutic evaluation than does the Sarcoma 180. These hopes were, in part, justified in that the efficacy of actinomycin was clearly demonstrated by two methods: daily determination of cells in the ascitic fluid.
fluid of individual treated mice and extension of median survival times of treated over control mice. It is a reasonable assumption that the comparison of the total number of cells in ascitic fluids from treated and control animals gives a good indication of the antitumor activity, because it was determined that 95 percent of the cells counted are tumor cells.

The foregoing observations indicate inhibitory effects of actinomycin for tumor cells and suggest its usefulness as a positive control in screening programs utilizing ascitic tumors.

**SUMMARY**

The ability of actinomycin D to exert a significant therapeutic effect on two types of ascitic tumors in the mouse is described. The inclusion of actinomycin as a positive control in antitumor screening is suggested.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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