The Effect of Prolonged Administration of 8-Azaguanine on the Growth of Transplanted Adenocarcinoma E0771*

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In 1949, Kidder and associates reported that the guanine analog 8-azaguanine inhibited the growth of adenocarcinoma E 0771 and of several other tumors in C57 black and other strains of mice (3). The inhibiting effects of 8-azaguanine in several types of adenocarcinoma have since been confirmed (1, 4, 5). The present study deals with the effects of prolonged administration on the growth of adenocarcinoma E 0771.

MATERIALS AND METHODS

Sixty-three mice of C57 black strain obtained from the Roscoe B. Jackson Laboratories in Bar Harbor, Maine, were used. The animals were maintained on a diet of Ralston Purina Fox Chow checkers and water ad libitum. 8-Azaguanine was obtained by courtesy of the Lederle Laboratories Division of the American Cyanamid Company. It was dissolved and administered twice daily by the procedure suggested by Kidder et al. (2). Adenocarcinoma E 0771 was transmitted by subcutaneous injection of a sterile suspension of tumor cells into the flank region of the animals.

The effect of prolonged administration of 8-azaguanine was studied in four groups of animals. The seventeen animals of Group 1 and the six animals of Group 2 were 5-6 weeks old at the time of tumor transmission, and the seven animals in Group 3 were 15-16 weeks old. Treatment in these three groups was begun the day the tumor could be palpated as a small nodule, i.e., between 6 and 13 days after transmission. In Group 4, unlike the preceding three, treatment was begun simultaneously with tumor transmission. Group 4 consisted of nine animals 7-8 weeks of age. The daily dose of 8-azaguanine was 1 mg. or about 55 mg/kg in Groups 1 and 4, and 1.4 mg. or about 79 mg/kg in Group 2. Group 3 was started with a dose of 1 mg., which was increased after 3-4 days to 1.4 mg., corresponding to 37 and 52 mg/kg, respectively.

Treatment and observations were continued until all or most of the animals died, except for Group 1. In this group, several animals with very rapidly and very slowly growing tumors were sacrificed.

Control observations on the rate of growth of adenocarcinoma E 0771 were made in nineteen animals 5-6 weeks of age and in nine animals 7-8 weeks of age. The animals were allowed to die spontaneously, except for some which were selected for sacrifice on the basis of extreme rates of tumor growth.

The degree of inhibition by 8-azaguanine was estimated as follows: In the control animals, the rate of growth was found to be different for tumors of different sizes. Since tumor size in the experimental animals varied over a wide range, it was necessary to take the size of the tumor into account. The growth rates for tumors of similar size in the control animals were therefore averaged. The effect of treatment was determined by expressing each daily growth rate as the percentage of the average rate at which tumors of the same size class were found to grow in the control animals. Tumor sizes were obtained daily, as the product of the three principal dimensions measured by caliper. Rates of growth were read from the plot of tumor size on a logarithmic scale against time on an arithmetic scale.

RESULTS

RATE OF TUMOR GROWTH IN UNTREATED ANIMALS

The size of the tumors came very close to the figures shown by Kidder et al. (2). There was only moderate variation in the rate of growth among different animals. With the increasing size of the tumor, the rate of its growth increased slightly at first; but when the size of the tumor increased to beyond 1,000 c.mm., the rate gradually decreased. The average rate rose from a 35 per cent increase per day for small sizes to 48 per cent for the size class 800–1,000 c.mm., then slowly declined to

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only 15 per cent per day for the class 8,000-15,000 c.mm. (Chart 1).

DECREASE IN GROWTH RATE PRODUCED BY 8-AZAGUANINE

Effect in the first 2 weeks.—Charts 2–4 show the influence of 8-azaguanine on Groups 1–3. During the first 2 weeks of treatment, the results were similar in the three groups in which the timing of treatment was the same. Retardation began with the first day after treatment; the inhibition grew more pronounced from day to day and reached a maximum in the second week. The size of the tumors in Group 1 was similar to that reported by Kidder, who employed animals of the same age and a similar dosage. On the tenth day, the rate of growth in these animals was only 13 per cent of that of the controls.

In Group 2, in which animals of the same age as those of Group 1 received a 40 per cent higher dose, the same degree of inhibition as in Group 1 was obtained: the growth rate on the seventh day was 29 per cent and on the eighth day 23 per cent of control growth, as compared to 30 per cent and 26 per cent in Group 1 for the same 2 days. The animals of this group died between 10 and 23 days after the beginning of treatment (Table 1); the animals with the lowest rates of tumor growth were the first to die.

Group 3 contained older animals, and a very low dosage was used initially, which was increased to that given Group 1. The inhibitory effect was very slight at first, and, even after the dosage was increased, it was not so pronounced as that in Group 1. However, it followed the same time course. Maximal inhibition occurred on the eleventh day.

In Group 4, in which treatment was begun on
the day of tumor transmission, not a single tumor developed during the first 2 weeks of treatment.

Effects of prolonged administration.—8-Aza-guanine decreased in effectiveness after the end of the second week. A progressive increase in rate of growth followed the maximal slowing seen on the 10th and 11th day. By the 24th day in Group 1 and the 21st day in Group 3, the tumors grew almost as rapidly as in untreated animals—i.e., at 82 per cent and 81 per cent of the control rate.

In Group 4, the first tumors were visible on the 18th day. By the end of the fourth week, the percentage of “takes” was the same as that seen in the other groups. The rates of growth of these tumors were as high as those measured in untreated animals.

Only in the two animals of Group 2 which survived beyond 15 days of treatment did 8-azaguanine retain some of its effectiveness in the third week. Rates of growth between 30 and 40 per cent of the control values were observed until shortly before the death of these animals.

The rapid growth of the tumors seen at the end of the third and beginning of the fourth week in Groups 1, 3, and 4 was followed by fluctuating rates which were of a similar pattern in all three groups. The growth rate in Group 1, after renewed depression, returned to 82 per cent of the control rate on the 28th day and again to 84 per cent on the 35th day. In Group 3, after the peak of 81 per cent on the 20th and 21st days, a second peak of 78-80-84 per cent was reached on days 28-30; in Group 4 a second peak of 76-78 per cent was noted on the 30th and 51st days.

### TABLE 1

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ANIMALS</th>
<th>SIZE OF TUMOR AT DEATH (c.c.m.)</th>
<th>SURVIVAL DURING TUMOR GROWTH (DAYS)*</th>
<th>SURVIVAL DURING TREATMENT (DAYS)</th>
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<td>Range Av.</td>
<td>Range Av.</td>
</tr>
<tr>
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<td>9</td>
<td>4.3-84.0 9.8</td>
<td>14-52 18</td>
<td>4-52 18</td>
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<tr>
<td>2</td>
<td>6</td>
<td>1.5-9.0 5.0</td>
<td>10-23 16</td>
<td>10-23 16</td>
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<tr>
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<td>7</td>
<td>0.8-15.6 7.7</td>
<td>10-34 27</td>
<td>10-34 27</td>
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<tr>
<td>4</td>
<td>9</td>
<td>0.7-11.4 6.7</td>
<td>4-32 18</td>
<td>33-57 41</td>
</tr>
</tbody>
</table>

*These columns represent the number of days from the appearance of a palpable nodule until the death of the animal.

**DISCUSSION**

Growth of Adenocarcinoma E 0771 in Control Animals

Our results are in good agreement with those reported by other authors. Gellhorn and collaborators (1) report quadrupling of tumor size in 5 days, which corresponds well with the growth rates observed in our smaller tumors. The tumor sizes reported by Kidder et al. (2) are slightly larger than those seen in our experiment, probably because more viable tumor cells are transmitted by the trocar method of transplantation than by the injection of a cell suspension.

The acceleration of growth rate from 35 to 48 per cent increase per day up to the size of 1,000 c.mm. is probably an artifact. The inclusion of two layers of skin with each tumor dimension as measured causes the growth rate to appear lower than it is with small tumors, but this error decreases progressively as the tumor increases in size. The progressively lower rates of growth of larger tumors might correspond to the progressively decreasing fraction of the total tumor mass which proliferates while the remainder undergoes cavity formation, necrosis, and peripheral ulceration.

Growth of Adenocarcinoma E 0771 in the Treated Animals

For the first 2 weeks of treatment, our results are in good agreement with the observations of
Kidder et al. (2) and of Gellhorn et al. (1). The effect of prolonged treatment with 8-azaguanine has not been reported previously for the E 0771 tumor.

For tumor 755 in C57 mice, Gellhorn and associates report continued inhibition of growth for periods up to 8 weeks, if treatment was begun the day after tumor transmission. These results appear to be in good agreement with those of Group 4 of the present investigation, if allowance is made for the differences caused by the different methods of tumor transmission. When tumor fragments are transmitted by the trocar method, the size of the fragment can be measured soon after inoculation. Inhibition of development by 8-azaguanine will be observed as unchanging size of the fragment. When tumors are transmitted by injection of a suspension of tumor cells, a failure to "take" will be observed for as long as the inhibition persists.

On the other hand, Gellhorn et al. report that in one group of five animals in which treatment was begun 10 days after transplantation and continued for 27 days there was "continued slow growth of the tumor without alteration in the growth curve." Treatment in this group was begun only a few days earlier than in our Groups 1 and 3. Gellhorn's observation of continued effectiveness of 8-azaguanine treatment for nearly 4 weeks is thus in disagreement with our findings for these two groups.

A study of the rate of incisor eruption in C57 black mice given similar doses of 8-azaguanine reveals a striking similarity between the effects on the growth rates of continuously growing teeth and those of tumors (3). Eruption of the incisors was depressed for the first 10–20 days. There was in nearly every instance a return to normal rates of eruption in the third and fourth week. Subsequently, the same peculiar periodic fluctuation in the growth rate of the incisors was observed that was noted for the tumors.

SUMMARY

8-Azaguanine was administered to 39 mice of C57 black strain bearing adenocarcinoma E 0771. The day-to-day degree of inhibition of tumor growth was measured by comparison with growth rates of the same tumor determined in control animals. Treatment was continued until all or most of the animals died.

1. When treatment was begun at the time the tumor had reached visible size, the rates of tumor growth progressively decreased during the first 10 days of treatment. With further treatment, a slow return to near control rates of growth took place during the fourth week.

2. When treatment was begun on the day of tumor transmission, tumors failed to develop for about 18 days. Subsequently, tumors showing nearly normal rates of growth were observed.

3. The effects on tumor growth described resemble the effects of 8-azaguanine on the growth rates of continuously growing teeth.

REFERENCES


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