The Effect of Colchicine on the Mitotic Activity of the Brown-Pearce Rabbit Epithelioma

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The purpose of this paper is to clarify if possible the sequence of events in the mitotic activity of the Brown-Pearce rabbit epithelioma after single and multiple doses of colchicine. These data show that the response of this rabbit tumor to colchicine varies considerably with the individual tumor, indicating that biopsy must be done to determine the mitotic response at any designated time interval after the administration of colchicine.

Reviewing the literature on the effect of colchicine on tumors, both animal and human, one is struck by the disagreement in the data and opinion presented by the various authors (1, 4, 15). These papers, in general, agree that in tumors of mice and rats there are more mitotic figures in the metaphase visible in histological sections after colchicine than in sections from untreated tumor (1, 4, 7, 10-13, 15). Most of such data are based upon groups of animals that have received single injections of the drug. Some have used toxic and others subtoxic doses (6, 7). Many report in vivo experiments (1, 4, 7, 10-15); others report in vitro experiments (5, 14).

**MATERIALS AND METHOD**

Rapidly growing, 4- to 5-week-old, Brown-Pearce rabbit epithelioma in young, susceptible, New Zealand white rabbits was used throughout these experiments. Small biopsy specimens from the tumor mass in the testicle were taken under sterile precautions at empirical intervals of 5 to 7 hours, after the injection of colchicine 1 into a series of rabbits bearing tumors of the same age and source. Biopsy specimens (1 cu. cm.) taken from central and peripheral parts of the tumor, having a minimal amount of necrosis, were fixed in formaldehyde solution, U.S.P., diluted 1:10, and Zenker's solution, and stained in the usual manner with hematoxylin and eosin. A cover slip was lined and cut to fit the microscope eyepiece. The projected image of the lines made possible a uniform counting of the cells over a constant area. Approximately 3,000 cells were counted per biopsy section. The following are typical experiments.

**RESULTS**

**Experiment I.**—Colchicine, dissolved in sterile saline, was injected subcutaneously into 17 rabbits, bearing 4-week-old Brown-Pearce rabbit epithelioma of uniform size (2.5 cm. x 4 cm.). Nine rabbits were given 0.25 mgm. per 100 gm. weight and 8 rabbits were given 0.125 mgm. per 100 gm. weight. Tumor material was removed at 5- to 7-hour intervals after one injection. Usually one testicle was removed from each of 2 rabbits at one interval, and the remaining testicle was removed at a subsequent interval. Representative specimens were taken from several areas for study; thus each rabbit supplied two testicles for study. The longest period before removing the testicle was 31 hours. Two rabbits receiving the larger dose died during the experiment. Another one with this large dose had a severe diarrhea.

The number of mitotic figures in the stained sections was counted. A maximum number of mitotic figures was obtained with both doses at the first interval of observation (Table I). There is no significant difference in the effect of the two doses used (0.125 to 0.25 mgm. per 100 gm. body weight). The number of mitotic figures fell progressively in the subsequent intervals (after 5 to 7 hours) except that there was an indication of a slight increase at 17 hours (Fig. 1). [Table 1: Average Counts of Mitotic Figures from Data of Experiment 1]

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Dose per 100 gm., in mgm.</th>
<th>Average count per 3,000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-7 hours</td>
<td>16-18 hours</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>194</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>.</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>183</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>.</td>
</tr>
</tbody>
</table>

* Trainee, National Cancer Institute.
† The colchicine was supplied by the Abbott Laboratories.
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(0.1 mgm. of colchicine per 100 gm. body weight). Specimens were taken from the testicles at 6, 17, and 31 hours. The maximum number of mitotic figures was observed at 6 hours, the other two intervals having less than one-third of this number. Fig. 2 shows the spread of the observations and the average effect graphically.

Experiment III.—The maximum effect of a single dose of colchicine in holding the tumor cell in the metaphase apparently occurs in the first interval (6 hours).

Table II: Period of Effect of 0.1 mgm. of Colchicine per 100 Gm. at 6-Hour Intervals from Data of Experiment III

<table>
<thead>
<tr>
<th>Hours</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Number of Observations</th>
<th>Standard Deviation of Mean</th>
<th>Significant</th>
</tr>
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<tr>
<td>Control</td>
<td>3.7</td>
<td>10</td>
<td>10</td>
<td>3.1</td>
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<tr>
<td>6</td>
<td>1.62</td>
<td>72</td>
<td>24</td>
<td>4.7</td>
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<tr>
<td>12</td>
<td>1.98</td>
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<td>22</td>
<td>9.1</td>
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<tr>
<td>18</td>
<td>1.79</td>
<td>19</td>
<td>47</td>
<td>4.3</td>
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<tr>
<td>24</td>
<td>1.35</td>
<td>64</td>
<td>24</td>
<td>12.8</td>
<td>Yes</td>
</tr>
<tr>
<td>30</td>
<td>1.32</td>
<td>44</td>
<td>16</td>
<td>11.0</td>
<td>No</td>
</tr>
</tbody>
</table>

Equal doses of 0.1 mgm. of colchicine at 6-hour intervals were given to study the cumulative effect. The maximum cumulative effect was produced by the second injection at 12 hours. Although there is considerable spread, the average figure at 6 hours is significantly different from and less than the average figure at 12 hours. The 28-hour average figure as compared to the 12-hour average figure is not significantly different when calculated and thus does not show an increase in mitotic activity. There is a significant fall after 18 hours (Table II; Fig. 3).

The distribution of mitotic figures in the sections taken at the same interval appears in Fig. 3. At 6 hours, the extremes range from 78 mitotic cells per 3,000 cells to 380 mitotic cells per 3,000 cells.

Experiment IV.—A further reduction in dosage to 0.05 mgm. of colchicine in repeated equal doses (in 6 hours) results in approximately half the number of mitotic figures obtained with the 0.1 mgm. dosage (Fig. 4). The maximum cumulative effect occurs during the 12th to 18th hour periods (Table III).

The cumulative effects with 0.1 and 0.05 mgm. of colchicine are roughly parallel (Fig. 5) although the number of mitotic figures is reduced to about 50 per
cent with the lower dose, for if the values obtained with the lower dose are multiplied by a factor of two, the spread and average almost superimpose those obtained with the higher dosage. Using a larger, single dose (above 0.1 mgm. in the previous experiments) did not show a significant increase in the number of mitotic figures (Table I). Thus 0.1 mgm. is apparently optimal for a single dose and apparently produces an optimal effect in repeated doses, judging from our limited data.

Experiment V.—Atropine has been considered antagonistic to colchicine (8). Three rabbits were given two doses of colchicine (0.1 mgm.) and atropine (0.4 mgm.) combined, with a 6-hour interval between the doses. Table IV shows the counts of the mitotic figures obtained. The atropine did not change the effect of the colchicine in the dose used during the 6-hour observation, which is the period of maximum mitotic figure count. Since all of the values are within the spread of the other data, the experiment was not carried further.

Discussion

The rabbit is quite resistant to colchicine. When colchicine is administered to a rabbit with an actively growing Brown-Pearce rabbit epithelioma, the cells in the metaphase stage of mitotic division are seen to be increased in number over the controls. The magnitude of this effect varies with the individual tumor. Tumors of the same age and from the same generation but in different sites, even in the same animal, do not give consistent results when treated with the same dosage of colchicine.

Colchicine administered in various doses shows considerable variation in its effect upon mitosis, roughly comparable to the dosage employed. The mean value obtained from single doses after 6 hours for 8 samples given more than 0.125 mgm. per 100 gm. is 188; that for 24 samples given 0.1 mgm. per 100 gm. being 162; and that for 12 samples given 0.05 mgm. per 100 gm. being 65.

The maximum number of mitotic figures in other animals was observed by other workers to have occurred at various periods extending over a range from 6 to 24 hours, and the increase varied from 12 to 38.2 percent in the colchicinized animals (5, 9-12).

In our experiments with the Brown-Pearce rabbit epithelioma, repeated doses of 0.1 mgm. or 0.05 mgm.
figures in the treated (150 in 3,000 cells) to the controls (37 to 3,000 cells) reached beyond 5.5 at the maximum (Table V). Obviously irradiation must destroy tumor cells which are not morphologically in the metaphase or other phases of obvious mitotic activity. This tumor is considered to be highly malignant yet normally only about 1 per cent of its cells are in a stage of mitotic activity. Colchicine elevates this number to individual maximum high values of 12 per cent. Since we have been unable to obtain a definite minimal lethal dose of roentgen radiation in vivo for this tumor, these data do not give sufficient encouragement for us to undertake the prolonged and extensive controlled experiment necessary for the study of the combined effects of colchicine and roentgen radiation at this time. Considering all of these features and the high toxicity and its unpredictable effects on the individual, we are led to suspect that colchicine is of little value as an additional aid to radiation therapy of this tumor.

CONCLUSIONS

1. Colchicine definitely causes an increase in the mitotic figure (metaphase) count in the Brown-Pearce rabbit epithelioma.

2. The optimal dose is 0.1 mgm. per 100 gm. body weight, and produces the maximum effect in single doses in approximately 6 hours after injection. The effect then gradually wears off.

3. With repetition of the dose at 6-hour intervals, the maximum effect occurs at 12 hours and declines thereafter. The average number of mitotic figures obtained with repetition of 0.1 mgm. per 100 gm. body weight was approximately twice that with 0.5 mgm. This numerical relationship was not obtained with single doses.

4. Although the average response in a group can be predicted for a given dosage, the response of the individual tumor varies greatly so that biopsy must be resorted to in order to determine the magnitude of its response to the drug.

5. The results were so unpredictable that a trial of the effect of colchicine and roentgen radiation did not seem feasible at this time.

REFERENCES


2. Unpublished work with S. Larson indicates that this is somewhere above 18,000 r single massive dose.


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