Use of Mouse Vaginal and Rectal Epithelium to Screen Antimitotic Effects of Systemically Administered Drugs

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SUMMARY

Effects on mitosis in mouse vaginal and rectal epithelium were used to screen antimitotic properties of drugs given i.p. Test drugs were given i.p. at time zero, podophyllin was injected at 2 hr, and the animals were killed at 8 hr. Both vagina and rectum were removed en bloc. Mitotic cells in the epithelia were counted histologically. The validity of the system was ascertained by testing drugs with known effects on the mitotic cycle. Untreated mice yielded low mitotic counts; mice receiving podophyllin alone had high counts; mice receiving cytosine arabinoside, methotrexate, nitrogen mustard, hydroxyurea, and 5-fluorouracil prior to podophyllin had low counts; and mice receiving either vinblastine followed by podophyllin or vinblastine alone showed high counts.

INTRODUCTION

The systems more commonly used to date for screening drugs for potential antineoplastic effectiveness have been various animal tumor systems and tissue culture preparations (3, 5, 11, 13). Tumor systems have provided information on the effectiveness of drugs in inhibiting the development and growth of transplanted cancers but have provided only limited information concerning the phase of the cell cycle most affected by a test drug. Tissue cultures may be utilized to determine cell cycle effectiveness for some chemical agents; but the exacting conditions required of cells in culture for survival, growth, and replication often complicate derivation of definitive information on the selective effects of a test material without performance of additional work in vitro or in animals. While information on the effects of chemical agents on the cell cycle can be sought in both systems through the use of radiolabeled materials, the techniques are relatively expensive, time consuming, and somewhat complex.

Insofar as the clinical value of a potential antineoplastic drug depends upon a favorable balance between cytotoxic effects on tumors and cytotoxicity on normal tissues, i.e., the therapeutic index, effects of a drug on normal tissues are important in preclinical evaluations of drug candidates. Normal vaginal and intestinal mucosae are characterized by rapid turnover times (2, 8, 12), a property utilizable in studying drug inhibition of cell replication. The tissues are readily accessible for monitoring effects of drugs in a routine screening procedure.

This study demonstrates that mouse vaginal and rectal epithelia provide simple, reliable, and inexpensive systems for detecting antimitotic effects of drugs.

MATERIALS AND METHODS

Virginal female CBA/Man, C57BL/10J, and CFW noninbred Swiss mice, 5 to 7 weeks old, were satisfactory for these studies. The i.p. doses of drugs (Table I) in most instances were 5 to 10 times the usual systemic human dose, except for podophyllin for which no human dose exists.

Test drugs and dosages were: podophyllin (USP), 80 mg/kg; cytosine arabinoside hydrochloride (Cytarabine), 375 mg/kg; sodium methotrexate, 37 mg/kg; mechlorethamine hydrochloride (nitrogen mustard, Mustargen), 0.75 mg/kg; hydroxyurea (Hydrea), 40 mg/kg; 5-fluorouracil, 100 mg/kg; and vinblastine sulfate (Velban), 25 mg/kg. All drugs were prepared in aqueous solution except for podophyllin for which the vehicle consisted of 45% propylene glycol, 45% distilled water, and 10% ethyl alcohol. Test drugs were injected i.p. at time zero, and podophyllin was injected i.p. 2 hr later. The animals were killed by cervical dislocation at 8 hr.

Both rectum and vagina were removed en bloc and fixed in 10% buffered formalin. Transverse sections were cut at 6 µ and stained with hematoxylin and eosin. An ocular micrometer permitted counting the number of mitotic cells per length of mucosa as measured along the basal cell layer of the vagina and the muscularis mucosae of the rectum. Scattered fields of mucosa were selected, and the number of mitoses in 3 separate 3-mm mucosal lengths was determined and expressed as mitoses/cm. The range of variability of repeated counts by this technique did not exceed 15% except in those instances in which median counts were very low.

The number of mitotic cells present after sequential administration of the test drug followed by podophyllin gives the desired information on the test drug for the following reason. Since podophyllin arrests cells in metaphase as they pass through mitosis, if the test drug given before i.p. podophyllin inhibits the cell cycle at G1, S, or G2, few cells will reach mitosis to be arrested in metaphase by podophyllin, and hence the number of metaphase cells will be small. If, on
the other hand, arrested mitotic cells are equal to or greater than the number found when podophyllin alone is given, it may be assumed either that the test drug is ineffective in inhibiting the cell cycle in the intermitotic phase or that the drug itself may be a metaphase-blocking agent. The drug in question is then readministered without podophyllin to determine which of these possibilities is applicable.

RESULTS

Vagina. All vaginal specimens were classified as being in either the estrogenic or progestational phase of the estrus cycle on the basis of histological characteristics. The vaginal mucosa of untreated mice during the phase of the estrus cycle primarily due to estrogen is characterized by stratification, keratinization, and a thick stratum corneum. The vaginal mucosa of an untreated mouse during the phase of the estrus cycle in which progesterone effects predominate is much thinner and is mucus secreting rather than keratinized.

Categorization of the mucosa as either estrogenic or progestational is important since the latter replicates only one-third to one-half as rapidly as the former (2, 12). Hence the number of cells arrested in metaphase by podophyllin given i.p. during the estrogenic phase is 2- to 3-fold the number arrested during the progestational phase.

The numerical data from animals with estrogenic vaginal epithelia are shown in Table 1. The number of mitotic cells/cm in mice receiving no drug at all is very low. Animals treated with podophyllin alone show high counts. Animals receiving cytosine arabinoside, methotrexate, nitrogen mustard, 5-fluorouracil, and hydroxyurea 2 hr prior to podophyllin show very low counts. In contrast, when vinblastine is given prior to podophyllin the count is high, and when vinblastine is given alone the count at 6 hr is again high, demonstrating its metaphase-arresting action.

Table 1
Mitotic counts in estrogenic vaginal epithelium of untreated mice and following i.p. administration of drugs with antimitotic effects

<table>
<thead>
<tr>
<th>Drug (i.p.)</th>
<th>No. of animals</th>
<th>Mitoses/cm</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>2–12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Podophyllin</td>
<td>23</td>
<td>67–436</td>
<td>237</td>
<td></td>
</tr>
<tr>
<td>Cytarabine-podophyllin</td>
<td>6</td>
<td>1–49</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Methotrexate-podophyllin</td>
<td>5</td>
<td>5–25</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mustargen-podophyllin</td>
<td>5</td>
<td>2–60</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Vinblastine-podophyllin</td>
<td>12</td>
<td>57–446</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td>13</td>
<td>40–864</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea-podophyllin</td>
<td>6</td>
<td>1–23</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil-podophyllin</td>
<td>7</td>
<td>5–142</td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Mitotic counts in progestation epithelium of untreated mice and following i.p. administration of drugs with antimitotic effects

<table>
<thead>
<tr>
<th>Drug (i.p.)</th>
<th>No. of animals</th>
<th>Mitoses/cm</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9</td>
<td>0–5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Podophyllin</td>
<td>7</td>
<td>17–144</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Cytarabine-podophyllin</td>
<td>2</td>
<td>2–6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Methotrexate-podophyllin</td>
<td>5</td>
<td>11–24</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mustargen-podophyllin</td>
<td>5</td>
<td>2–39</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Vinblastine-podophyllin</td>
<td>3</td>
<td>31–120</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td>6</td>
<td>22–108</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea-podophyllin</td>
<td>3</td>
<td>10–21</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil-podophyllin</td>
<td>3</td>
<td>10–21</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Chart 1. Mitotic counts in vaginal epithelium following i.p. administration of drugs shown. 5 FU, 5-fluorouracil.
The data of this study suggest that mouse vaginal and rectal epithelia can provide systems by which to screen antimitotic properties of other drug candidates. Neither tissue seems more susceptible than the other to the effects of the drugs tested. However, rectal tissue displays a higher rate of replication, as judged by higher mitotic counts following administration of podophyllin, and is similar in this regard to colon mucosa (8). Its high replication rate may facilitate a more sensitive evaluation of antimitotic effects of drugs than that permitted by vaginal mucosa.

It may be argued that in vitro cell culture systems, by virtue of a more defined environment of the cells, permit an uncomplicated evaluation of effects of drugs on the cell cycle. This is not necessarily so because of the fastidious environmental requirements of these systems. Moreover, antimitotic effects of drugs clearly identifiable in tissues of an intact animal seem more pertinent to the eventual clinical use of these drugs.

REFERENCES

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