Antineoplastic Effect of Chlorpromazine in Chemical Carcinogenesis in the Hamster Cheek Pouch

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SUMMARY

Hamster cheek pouches were treated topically with 9,10-dimethyl-1,2-benzanthracene (DMBA) alone, in combination with chlorpromazine (CPZ), or following pretreatment with CPZ.

After 9 weeks of application of 1.2% CPZ and 0.5% DMBA simultaneously, only one microscopic focus of intraepithelial carcinoma was present in one of the six animals, whereas in the six animals treated with DMBA alone, nine atypical papillomas and three invasive carcinomas were present. After 12 weeks, only one carcinoma and one atypical papilloma were found in two of the six animals treated with CPZ and DMBA, in contrast to the finding of 18 carcinomas and 8 atypical papillomas in the pouches after DMBA alone. The dose-related effect of CPZ was demonstrated when, in a similar procedure, with 0.6% CPZ, only partial inhibition of carcinoma formation was obtained after 9 and 12 weeks.

When the pouches were pretreated with topical 1.2% CPZ for 12 weeks prior to DMBA application, almost complete inhibition of tumor formation was obtained.

The antineoplastic action of CPZ is reviewed and the membrane and lysosomal stabilizing properties and dose relation of this drug are discussed. It is suggested that the inhibitory effect of CPZ may be related to decreased permeability of cellular and subcellular membranes, resulting in decreased permeation of the carcinogen into the cellular structures. It is also possible that stabilization of lysosomes is achieved, with decreased release of lysosomal enzymes known to influence nuclear activity and with consequent delay in cell division.

INTRODUCTION

In previous studies from this laboratory, chemical carcinogenesis in the hamster cheek pouch was promoted by the administration of the membrane and lysosomal labilizers vitamin A palmitate (15, 20) and stilbestrol diphosphate (19). On the other hand, cortisone, a known membrane-stabilizing agent, produced the reverse effect when administered topically (21).

CPZ is another drug known to influence the function of biological membranes including lysosomes (11, 12, 17), and this effect appears to be dose dependent (9, 17). In preliminary studies (14), a 1.2% solution of CPZ applied topically to the hamster cheek pouch mucosa during chemical carcinogenesis inhibited tumor formation.

In the present study, the effects of different concentrations of topical CPZ applied during and before chemical carcinogenesis to the hamster cheek pouch are recorded.

MATERIAL AND METHODS

A total of 86 male Syrian hamsters were used. The animals were of the same local strain as that used in previous studies (14, 15, 19–21). Their age was 1.5 to 2 months at the start of the experiment, and their weight was 55 to 65 g. DMBA and CPZ were dissolved in liquid paraffin on a weight-to-volume basis. The solutions were applied 3 times per week to the right cheek pouches with a No. 4 camel’s hair brush. The brush was dipped into the solution and the excess was allowed to drip off. The brush was inserted into the pouch, which was then stroked firmly. The animals were housed 3 per cage, and they received Purina laboratory chow and tap water ad libitum. No spontaneous deaths occurred throughout the experiment.

When the animals were sacrificed, autopsies were performed, and both cheek pouches, regional lymph nodes, and internal organs were examined histologically after fixation in Bouin’s fluid and paraffin embedding. The treatment schedules of the different groups are given in Table 1.

In the light of the results of previous studies (14, 15, 19–21), which demonstrated that paraffin oil produces no effect during DMBA carcinogenesis in the hamster cheek pouch, control groups receiving paraffin oil only after application of CPZ were not included.

RESULTS

During the macroscopic examination of the cheek pouches, the tumors were counted, and their nature was then determined histologically. Multiple sections were also taken from macroscopically nontumorous areas of the mucosa. The neoplastic lesions encountered were classified, as in previous studies (Ref. 19; this paper contains illustrations of the various

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1 Supported by a grant from the Research Promotion Fund of the Histadrut (General Federation of Jewish Labour).
2 Supported by a grant from Mr. Michael Kerber, Toronto, Canada.
Received March 3, 1972; accepted June 1, 1972.

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The abbreviations used are: CPZ, chlorpromazine; DMBA, 9,10-dimethyl-1,2-benzanthracene.
Table 1
Treatment schedule of buccal pouches of Syrian golden hamsters receiving 3 weekly applications of DMBA and/or CPZ in liquid paraffin, in different combinations, concentrations, and sequences.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Treatment</th>
<th>Period</th>
<th>Treatment</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>DMBA 0.5%</td>
<td>Weeks 1—9</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ia</td>
<td>6</td>
<td>DMBA 0.5%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>DMBA 0.5% and CPZ 0.6%</td>
<td>Weeks 1—9</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IIa</td>
<td>6</td>
<td>DMBA 0.5% and CPZ 0.6%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>DMBA 0.5% and CPZ 1.2%</td>
<td>Weeks 1—9</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IIIa</td>
<td>6</td>
<td>DMBA 0.5% and CPZ 1.2%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>CPZ 0.6%</td>
<td>Weeks 1—9</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>IVa</td>
<td>3</td>
<td>CPZ 0.6%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>CPZ 1.2%</td>
<td>Weeks 1—9</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Va</td>
<td>3</td>
<td>CPZ 1.2%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>VI</td>
<td>16</td>
<td>CPZ 1.2%</td>
<td>Weeks 1—12</td>
<td>DMBA 0.5%</td>
<td>Weeks 13—24</td>
</tr>
<tr>
<td>VII</td>
<td>16</td>
<td>None</td>
<td>Weeks 1—12</td>
<td>DMBA 0.5%</td>
<td>Weeks 13—24</td>
</tr>
<tr>
<td>VIII</td>
<td>6</td>
<td>CPZ 1.2%</td>
<td>Weeks 1—12</td>
<td>None</td>
<td>Weeks 13—24</td>
</tr>
</tbody>
</table>

a S, sacrifice.

Table 2
Incidence of malignant and premalignant tumors in hamster cheek pouches treated topically with DMBA alone, with a combination of DMBA and CPZ, or with DMBA with or without pretreatment by CPZ.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Animals with carcinoma</th>
<th>Total no. of carcinomas</th>
<th>Av. no. of carcinomas/pouch</th>
<th>Animals with atypical papillomas</th>
<th>Total no. of atypical papillomas</th>
<th>Av. no. of atypical papillomas/pouch</th>
<th>Intraepithelial carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simultaneous CPZ and DMBA 9 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0.5</td>
<td>4</td>
<td>9</td>
<td>1.5</td>
<td>++</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0.3</td>
<td>2</td>
<td>2</td>
<td>0.3</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+ (in 1 animal)</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>6</td>
<td>18</td>
<td>3.0</td>
<td>6</td>
<td>8</td>
<td>1.3</td>
<td>++</td>
</tr>
<tr>
<td>Va</td>
<td>16</td>
<td>5</td>
<td>8</td>
<td>1.3</td>
<td>6</td>
<td>10</td>
<td>1.7</td>
<td>+ (in 2 animals)</td>
</tr>
<tr>
<td></td>
<td>Pretreatment with CPZ 12 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+ (in 2 animals)</td>
</tr>
<tr>
<td>VII</td>
<td>16</td>
<td>13</td>
<td>34</td>
<td>2.1</td>
<td>12</td>
<td>21</td>
<td>1.3</td>
<td>++</td>
</tr>
</tbody>
</table>

lesions), as –: benign epithelial hyperplasia with hyperkeratosis, intraepithelial carcinoma, atypical papillomas, and invasive squamous-cell carcinomas. The latter 2 lesions could be counted accurately. However, since intraepithelial carcinoma did not always present as a tumor macroscopically, the frequency of these lesions had to be estimated from multiple histological sections. Thus, the findings had to be expressed as – when these lesions were not found, as + when only a few of these lesions were encountered in some animals of a group, or as ++ when many lesions were present in each animal.

The cheek pouches in Groups IV, IVa, V, Va, and VIII were within normal limits. The results in the other groups are summarized in Table 2 and compared in Chart 1.

No pathological changes were found in the nontreated cheek pouches or in the internal organs. Metastases were never found in the regional lymph nodes, nor in any other organs.

DISCUSSION

In the present study, the carcinogenic effect of DMBA on the hamster cheek pouch was partly suppressed by simultaneous topical administration and almost completely inhibited by preceding local treatment with 1.2% CPZ. This effect of the drug was dose related, since 0.6% CPZ caused only partial inhibition of the carcinogenic effect of DMBA, reducing the number of carcinomas to half the expected number, whereas premalignant lesions developed as frequently as in animals treated with DMBA alone.
The promotion of chemical carcinogenesis in the hamster cheek pouch by membrane-labilizing agents, encountered in previous studies (15, 19, 20), has been tentatively attributed to the effect of these agents on cell membranes or lysosomes and, consequently, on nuclear activity (2, 3, 13, 16). Some authors have suggested that lysosomal labilization may be an important factor in the initiation of cell division and carcinogenesis (1—3, 13), achieved either by the alteration of nuclear material by lysosomal enzymes or by the release of cells from mitotic inhibition via the action of these enzymes (2, 3). An alternative explanation for this enhancing phenomenon may be the increased permeability achieved by these agents, facilitating easier penetration of the carcinogen and consequent potentiation of its carcinogenic effect.

An inhibiting effect of CPZ on tumorigenesis has previously been recorded by others (4, 5, 7, 8, 10, 22). However, the mechanism of the antineoplastic action of CPZ has not been ascertained.

Evidence has been presented both in vivo and in vitro, showing the stabilization by CPZ of lysosomal and other biological membranes (11, 12). In these studies, CPZ antagonized the lysosomal labilizing effect of vitamin A (11), and it was suggested that this action may well explain the protective effect of phenothiazine compounds after irradiation and endotoxic and hemorrhagic shock. In general, CPZ appears to decrease cell permeability; however, some workers (9) have claimed that large doses of this drug may cause increased cell permeability. These results and the work of others (17) have shown the membrane effect of CPZ to be dose related.

In the light of the above data, it seems feasible to suggest that the inhibitory action of CPZ during carcinogenesis may be related to decreased permeability of the cell membrane, resulting in less effective penetration of the carcinogen into the cells and subcellular structures and possibly also in stabilization of lysosomal membranes, with decreased liberation of lysosomal enzymes that play a role in the early stages of cell division and carcinogenesis. The latter hypothesis is supported by evidence showing that CPZ inhibits incorporation of tritiated thymidine into DNA (23) and suppresses DNA synthesis in the bone marrow granulocyte series, causing delayed cell division (18). On the other hand, it is known that in certain tumors there is preferential accumulation of CPZ, which may produce toxic effects on the metabolism of the cells concerned (6, 22). Thus, it is also possible that CPZ may cause a specific metabolic action on tumors, resulting in inhibition of tumor growth.

REFERENCES

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