The Effect of Topical Vitamin A on Papillomas and Intraepithelial Carcinomas Induced in Hamster Cheek Pouches with 9,10-Dimethyl-1,2-benzanthracene

A. Polliack and I. S. Levij
Department of Pathology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

SUMMARY

Foci of intraepithelial carcinoma and benign papillomas up to 2 mm in diameter were present in the cheek pouches of all 6 hamsters sacrificed immediately after completion of the local treatment with 0.5 percent 9,10-dimethyl-1,2-benzanthracene (DMBA) in paraffin during a period of 6 weeks. Among 6 hamsters treated similarly but sacrificed 2 months after discontinuation of the local application of DMBA, only 3 showed benign papillomas, and no carcinomas were present. Of the 6 animals receiving DMBA for 6 weeks, followed by local application of paraffin for 2 months, only 1 showed a papilloma with intraepithelial carcinoma, and in the remaining 5 animals no tumors were found. All 6 animals receiving similar treatment with DMBA during 6 weeks, followed by topical administration of 10 percent vitamin A palmitate in paraffin during 2 months, showed infiltrating squamous cell carcinomas.

The epithelial changes induced with DMBA during 6 weeks thus regressed partially when application of the carcinogen was discontinued. However, subsequent treatment of these lesions with vitamin A induced an increase in size and malignancy. This effect of vitamin A may be related to increased permeability of cellular and subcellular membranes with resulting increased permeation of the carcinogen into cellular structures. However, since the administration of DMBA was discontinued prior to the application of vitamin A, the findings probably resulted from a direct effect of vitamin A on metabolic processes in epithelial cells which had previously been altered by DMBA.

INTRODUCTION

The effects of vitamin A on the differentiation and keratinization of epithelial cells are well established (9, 23). Vitamin A deficiency is known to cause squamous metaplasia of mucus-secreting epithelium in some animals (24, 28). Clinically, leukoplakia in various sites and dermatologic disorders characterized by hyperkeratosis have been treated with partial success with vitamin A (19, 22, 26). In addition, vitamin A has been shown to inhibit the induction and to retard the growth of experimental tumors. The effect of vitamin A varies in different animals and is also dependent on the site of application. Vitamin A inhibited tumors induced with 9,10-dimethyl-1,2-benzanthracene (DMBA) in the cervix and vagina of hamsters, but it had no protective effect in the perineal skin of these animals (5). Vitamin A inhibited the growth of viral-induced Shope rabbit skin papillomas (18) and reduced the number and size of DMBA-induced Rhino mouse skin papillomas (6).

However, in studies reported previously, topical excess of vitamin A potentiated DMBA carcinogenesis in the hamster cheek pouch. When the vitamin was administered simultaneously with the carcinogen, malignant epithelial tumors developed earlier, in slightly increased numbers, and reached a larger size than in animals treated with DMBA alone (14, 20). Furthermore, administration of excess vitamin A increased the incidence of avian leukemia (17).

In view of these data we endeavored to determine the effect of topical vitamin A on the epithelial changes induced by a preceding short-term treatment of the hamster cheek pouch with DMBA.

MATERIALS AND METHODS

The right cheek pouches of 24 male Syrian golden hamsters (weight 55–65 gm) were painted 3 times per week for 6 weeks with a 0.5 percent solution of DMBA in liquid paraffin. After discontinuation of DMBA, 6 animals (Group I) were sacrificed immediately. The remaining 18 animals were sacrificed 2 months later. During this 2-month period, the right cheek pouches of 6 animals (Group II) were painted 3 times per week with a 10 percent solution of vitamin A palmitate in liquid paraffin. Vitamin A palmitate, 1.7 million U/gm, was obtained from E. Merck AG, Darmstadt. The right cheek pouches of 6 animals (Group III) were painted 3 times per week with a 10 percent solution of vitamin A palmitate in liquid paraffin. Vitamin A palmitate, 1.7 million U/gm, was obtained from E. Merck AG, Darmstadt. The right cheek pouches of 6 animals (Group IV) were left without treatment throughout the 2-month period. The animals received Purina laboratory chow and tap water ad libitum.

Autopsies were performed and both cheek pouches, regional lymph nodes, and internal organs were examined histologically. No spontaneous deaths occurred throughout the experiment.

1Supported by Grant 34.630 of the Joint Research Fund, Hebrew University-Hadassah Medical School.

Received December 1, 1967; accepted October 1, 1968.
RESULTS

The findings are summarized in the Table.

Group I. In all treated pouches there was irregularity of the mucosa with contraction of the distal end of the pouch, and 1 or 2 papillary tumors, 1–2 mm in diameter, were found in every animal (Fig. 1).

Histologically there was diffuse marked hyperkeratosis and focal acanthosis, which was at times irregular, with mild epithelial atypicality. There was rete peg formation and inflammation with fibrosis of the lamina propria. In addition, small foci of intraepithelial carcinoma were present in all pouches, with irregular acanthosis, severe cellular atypicity, and loss of polarity, but without invasive growth (Fig. 2). The macroscopic tumors were all benign papillomas, some of which showed mild epithelial atypicality. No infiltrating carcinoma was found in any of the animals.

Group II. In all treated pouches 2 to 6 irregular tumors were present, up to 8 mm in diameter (Fig. 3). The nontumorous mucosa was irregular.

Histologically all tumors were markedly pleomorphic squamous cell carcinomas with extensive infiltrative growth (Fig. 4). Many tumors were partially necrotic, ulcerated, and hemorrhagic. Occasional tumor cells showed ballooning and vacuolization of the cytoplasm. Many tumor cells were present at the surface of the tumors, and there was less mature keratin present than in Group I. In addition, multiple foci of intraepithelial carcinoma were found. Other areas showed acanthosis with less keratinization than in Group I.

Group III. In all treated pouches there was irregularity of the mucosa. One animal showed a single papillary tumor, 3 mm in diameter, in the proximal end of the pouch (Fig. 5). No tumors were seen in the remaining 5 animals.

Histologically the general finding in all pouches was diffuse, marked epithelial atrophy (Fig. 9). The tumor seen microscopically was a papilloma with areas of marked epithelial atypicality and loss of polarity, but without invasive growth. These areas were regarded as intraepithelial carcinomas (Fig. 6). In the grossly nontumorous mucosa there were occasional foci of irregular acanthosis with moderate cellular atypicality, but without loss of polarity. These foci were therefore not regarded as intraepithelial carcinoma. No infiltrative growth was present.

Group IV. In all treated pouches there was irregularity of the mucosa, with contraction of the distal end. Solitary papillary tumors, 1–2 mm in diameter, were seen in 2 pouches (Fig. 7). One animal showed 2 tumors of the same dimensions. No tumors were seen in the remaining 3 animals.

Histologically all pouches showed areas of epithelial atrophy with hyperkeratosis. There was focal acanthosis, with occasional cellular atypicality (Fig. 8). Less pleomorphism was present than in Groups I or II, and no loss of polarity was seen. These lesions were not regarded as intraepithelial carcinomas. The macroscopic tumors were all benign papillomas, 1 of which showed foci of moderate cellular atypicality. There were no intraepithelial or infiltrating carcinomas.

DISCUSSION

From previous experiments (15, 20) it is known that in the early stages of DMBA carcinogenesis in the hamster cheek pouch there is inflammation, necrosis, and reactive epithelial

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Macroscopic appearance</th>
<th>Number of tumors</th>
<th>Diameter of tumors (mm)</th>
<th>Microscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA for 6 weeks; killed immediately</td>
<td>Irregular mucosa; small papillary tumors in all animals</td>
<td>1–2</td>
<td>1–2</td>
<td>Diffuse hyperkeratosis and focal acanthosis; benign papillomas; foci of intraepithelial carcinoma.</td>
</tr>
<tr>
<td>II</td>
<td>DMBA for 6 weeks, followed by vitamin A for 2 months</td>
<td>Irregular mucosa; multiple large tumors in all animals</td>
<td>2–6</td>
<td>5–8</td>
<td>Anaplastic infiltrating squamous cell carcinomas; multiple foci of intraepithelial carcinoma.</td>
</tr>
<tr>
<td>III</td>
<td>DMBA for 6 weeks, followed by paraffin for 2 months</td>
<td>Irregular mucosa; small papillary tumor in 1 animal</td>
<td>0–1</td>
<td>3</td>
<td>Epithelial atrophy and focal acanthosis with occasional cellular atypicality; papilloma with intraepithelial carcinoma.</td>
</tr>
<tr>
<td>IV</td>
<td>DMBA for 6 weeks; no further treatment; killed 2 months later</td>
<td>Irregular mucosa; small papillary tumors in 3 animals</td>
<td>0–2</td>
<td>1–2</td>
<td>Epithelial atrophy and hyperkeratosis; focal acanthosis with occasional cellular atypicality; benign papillomas in 3 animals.</td>
</tr>
</tbody>
</table>

Results of local treatment of hamster cheek pouches with DMBA, followed by a treatment-free interval or by local application of vitamin A or paraffin. (Each group consisted of 6 animals). DMBA, 9,10-dimethyl-1,2-benzanthracene.
hyperplasia with marked hyperkeratosis. This is followed by the development of benign papillomas, intraepithelial carcinomas (after 6 to 8 weeks), and eventually infiltrating squamous cell carcinomas (after 8 to 11 weeks).

In the present study there was hyperkeratosis and focal acanthosis with epithelial atypicality after 6 weeks of treatment with DMBA. In addition, there were benign papillomas, and in all pouches intraepithelial carcinomas were found.

When the pouches were left untreated for 2 months after cessation of DMBA application, no infiltrating carcinomas developed. On the contrary, there were fewer areas of epithelial hyperplasia, and histologically these showed no signs of malignancy. Thus, partial regression of the epithelial changes had taken place after the treatment with DMBA was discontinued.

The findings in the animals treated for 2 months with paraffin after discontinuation of DMBA were basically the same as those in the animals receiving no further treatment, but less macroscopic tumors were found. The presence of areas of intraepithelial carcinoma in the single papilloma found suggests lack of spontaneous regression of a DMBA induced premalignant lesion.

However, when lesions induced with DMBA during 6 weeks were subsequently treated with a mild topical excess of vitamin A (10 percent) for 2 months, large anaplastic carcinomas with marked invasive growth and many foci of intraepithelial carcinoma were found.

In various experimental models utilizing otherwise untreated epithelium, vitamin A in mild excess caused epithelial changes, including hyperplasia, mild atypicality, disorientation of the basal layer, rapid epidermal proliferation, an increased mitotic index, and mucoid metaplasia (2, 4, 10-13, 25).

In previous studies, local application of vitamin A for 9–12 weeks caused epithelial hyperplasia and atypicality in the hamster cheek pouch (14, 20, 21).

The rapid development of tumors during application of vitamin A, in epithelium previously treated with DMBA, may be related to the above-mentioned epithelial changes resulting from a mild excess of vitamin A.

Vitamin A alters the permeability of cellular and subcellular membranes (7, 16) and promotes the release of lysosomal enzymes (1, 3, 8, 27). This may cause more effective permeation of a carcinogen into cells and subcellular structures, resulting in rapid development of tumors when vitamin A is applied simultaneously with DMBA (14, 20). However, in the present study vitamin A was applied after cessation of DMBA. Therefore it seems more likely that the present findings resulted from the direct effect of vitamin A on epithelium which had previously been altered by DMBA.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Gad Ganem and Miss Lidia Scalozub for their technical assistance.

REFERENCES


Fig. 1. Cheek pouch of hamster sacrificed immediately after completion of local treatment with 9,10-dimethyl-1,2-benzanthracene (DMBA) during 6 weeks, showing irregularity of the mucosa, contraction of the distal end of the pouch, and a small papillary tumor.

Fig. 2. From cheek pouch shown in Fig. 1. Area of intraepithelial carcinoma with pleomorphism and loss of polarity but without infiltrative growth. H & E, x 270.

Fig. 3. Check pouch of hamster sacrificed after treatment with 9,10-dimethyl-1,2-benzanthracene (DMBA) during 6 weeks, followed by the application of vitamin A during 2 months, showing irregularity of the mucosa, and 2 large tumors in the distal end of the pouch.

Fig. 4. From check pouch shown in Fig. 3. Infiltrating keratinizing squamous cell carcinoma with necrosis, ulceration and hemorrhage. H & E, x 43.

Fig. 5. Cheek pouch of hamster sacrificed after treatment with 9,10-dimethyl-1,2-benzanthracene (DMBA) during 6 weeks, followed by paraffin for 2 months, showing irregularity of the mucosa and a papillomatous tumor. This was the only tumor found in this group of animals.

Fig. 6. Area of intraepithelial carcinoma from tumor shown in Fig. 5. This was the most advanced lesion found in this group. H & E, x 270.

Fig. 7. Check pouch of hamster sacrificed 2 months after the completion of treatment with 9,10-dimethyl-1,2-benzanthracene (DMBA) during 6 weeks without treatment in the intervening period, showing irregularity of the mucosa, slight contraction of the distal end of the pouch, and 2 small papillary tumors.

Fig. 8. From cheek pouch shown in Fig. 7. Area of acanthosis with cellular atypicality and occasional dyskeratosis but with preserved polarity. This was the most advanced lesion found in this group. H & E, x 270.

Fig. 9. From cheek pouch of hamster treated with 9,10-dimethyl-1,2-benzanthracene (DMBA) for 6 weeks, followed by paraffin for 2 months, showing diffuse epithelial atrophy. This was the general appearance of the mucosa in both Groups III and IV. H & E, x 270.
The Effect of Topical Vitamin A on Papillomas and Intraepithelial Carcinomas Induced in Hamster Cheek Pouches with 9,10-Dimethyl-1,2-benzanthracene

A. Polliack and I. S. Levij


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="http://cancerres.aacrjournals.org/content/29/2/327">http://cancerres.aacrjournals.org/content/29/2/327</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E-mail alerts</th>
<th>Sign up to receive free email-alerts related to this article or journal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, contact the AACR Publications Department at <a href="mailto:permissions@aacr.org">permissions@aacr.org</a>.</td>
</tr>
</tbody>
</table>