Cortisone and Hamster Buccal Pouch Carcinogenesis

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Summary

Ninety male Syrian hamsters, 3 months of age, were divided into 6 equal experimental groups of 15 animals. Two groups served as controls for 8- and 16-week experimental periods. In the remaining 4 groups, the right buccal pouch was painted 3 times weekly with 9,10-dimethyl-1,2-benzanthracene (DMBA) and sacrificed at 8 and 16 weeks. One 8-week and one 16-week experimental group were also injected 3 times weekly with 2.0 mg of cortisone acetate. It was found that in those animals receiving cortisone as well as DMBA applications, the development of initial dysplasia and of buccal pouch carcinomas was hastened. Although the tumors induced in the cortisone-DMBA animals were histologically not more anaplastic than those in the DMBA animals, invasion of underlying connective tissue and muscle was more extensive and the tumors were considerably larger in size. Metastasis to regional lymph nodes was not observed in any of the groups.

Introduction

The relationship between corticosteroid hormones and carcinogenesis has received considerable attention in recent years. Since the initial work of Sugiura (17), a diversity of experimental results has been reported, and the site studied most comprehensively has been the mouse skin. An augmentation of the effect of carcinogenic chemical agents upon epidermis has been reported by Sulzberger et al. (18) and by Spain et al. (16). Sulzberger and co-workers found that after 6 weeks of methylcholanthrene application to the skin of mice, papillomas developed in 69% of animals injected daily with cortisone, and in 48% of uninjected animals (18). A retardation in carcinogenesis has been reported by Engelbreth-Holm and Asboe-Hansen (4), and by Ghadially and Green (5). No appreciable effect of cortisone on the development of squamous cell carcinoma of mouse skin was noted by Gillman et al. (6). Baserga and Shubik (2) found that cortisone did not inhibit the growth rate of induced skin tumors or subcutaneous sarcomas in experimental animals.

Experimental technics have varied considerably in the various experiments. The dosage of cortisone has ranged from 0.2 mg daily (4) to 2 mg daily (16) in mice. Administration of cortisone has included s.c., i.p., and i.m. as well as local injections. Topical application of cortisone has been utilized by Ghadially and Green (5), who found that it inhibited the development of chemically induced papillomas in mouse skin.

Cortisone, injected locally, has been found to inhibit the development of chemically induced neoplasms of hamster submandibular glands (11). However, systemically administered cortisone has been found by Anbari et al. (1) to enhance the process of chemical carcinogenesis of rat salivary glands so that carcinomas developed more rapidly in the submandibular gland after implantation of pellets of dimethylbenzanthracene. Species specificity would probably tend to explain the different results in salivary gland carcinogenesis, since it has been shown that sarcomas develop in hamster submandibular gland, while carcinomas develop in rat submandibular gland in response to carcinogenic chemical action.

The effect of systemically administered cortisone on the development of experimental mucosal tumors has not been described. Chemically induced carcinomas of the hamster buccal pouch were first reported by Salley (12). The technic was further refined by Morris (8) and has been widely used in recent years (13, 14). Chemical carcinogenesis of hamster buccal pouch has been shown to be augmented by the local application of croton oil together with the carcinogenic chemical agent. Sabes et al. (11) reported some effect on carcinogenesis when cortisone was applied locally to hamster pouch mucosa prior to the application of the carcinogen. The latent period was not significantly altered, but the tumor yield appeared somewhat higher in the cortisone painted animals.

Since the role of cortisone in mucosal carcinogenesis could be studied more definitively by systemic administration throughout the period of neoplastic transformation, an experiment was set up to determine the effect of cortisone on the time required before neoplastic tissue changes were apparent, on the number and size of tumors induced, and on the degree of anaplasia in the induced tumors.

Materials and Methods

Ninety male Syrian hamsters (Cricetus auratus), 3 months of age were used as experimental animals and were divided into 6 groups with 15 animals in each group.

- **Group A**: Control, 8-week experimental period.
- **Group B**: DMBA painted, 8-week experimental period.
- **Group C**: DMBA painted, cortisone injected, 8-week experimental period.
- **Group D**: Control, 16-week experimental period.
- **Group E**: DMBA painted, 16-week experimental period.
- **Group F**: DMBA painted, cortisone injected, 16-week experimental period.

Received May 26, 1966; accepted June 27, 1966.
The right buccal pouches of animals in Groups B, C, E, and F were painted 3 times weekly with 0.5% DMBA (9,10-dimethyl-1,2-benzanthracene) in heavy mineral oil USP. The carcinogen was applied with a number 4 sable brush. Animals in Groups C and F, in addition to the application of DMBA, received a s.c. injection into the groin of 2 mg of cortisone acetate. The cortisone was also administered 3 times weekly during the course of the experiment. The animals of Groups A, B, and C were sacrificed with ether at the end of an 8-week experimental period. The animals of Groups D, E, and F were sacrificed with ether at the end of a 16-week experimental period. At the conclusion of the experimental period and following sacrifice of the animal, the right buccal pouch was removed and fixed in 10% formalin. Autopsies were performed on all animals and organs removed for study were adrenals, liver, kidney, spleen, heart, lungs, and salivary glands with attached cervical lymph nodes.

The tissues were sectioned in paraffin and stained with hematoxylin and eosin.

Results

Gross Observations

No gross tumors were observed in the right pouches of animals in Group B, although the surface texture tended to be more rough and pebbled than in the control Group A. In Group C there were a number of small tumor masses grossly visible on the pouch surface of several experimental animals.

In Groups E and F, most right pouches presented gross evidence of tumor development. In Group E the tumors tended to be smaller and more discrete than in Group F, and the pouches were freely movable. In many animals of Group F, the neoplasms appeared to involve the entire pouch and the pouch itself was fixed to adjacent tissues and could not be everted. These larger tumors also appeared to have more areas of necrosis and ulceration.

Microscopic Observations

Eight-week experimental period. Group A—control animals: In the control group of hamsters, the buccal pouch epithelium was characterized by a well-defined stratum corneum, but there was no evidence of hyperkeratosis or dysplasia. Chronic inflammatory infiltration of the underlying corium was absent.

Group B—DMBA-painted animals: The buccal pouch epithelium in this group of hamsters presented a variety of alterations leading to malignancy. In only 1 animal was there an invasive epidermoid carcinoma, but carcinoma in situ was found in 5 animals, and hyperkeratosis with dysplasia was observed in 6 animals. The carcinoma in situ lesions were characterized by variation in cell size, shape, and chromaticity, by altered nuclear-cytoplasmic ratio, and by the presence of numerous mitoses. Bizarre mitoses and bizarre cell forms were not commonly observed. In the carcinoma in situ lesions, the surface was usually parakeratotic rather than hyperkeratotic. Three animals presented hyperkeratosis.

Group C—DMBA-painted, cortisone-injected animals: Most animals in this group presented invasive epidermoid carcinoma, although 3 animals demonstrated only carcinoma in situ lesions and 4 animals demonstrated hyperkeratosis with areas of dysplasia. The epidermoid carcinomas were well-differentiated lesions with numerous areas of keratinization. Hyperchromatic and large nuclei were commonly observed, but bizarre cell forms and bizarre mitoses were infrequently noted. Invasion into the underlying corium was a feature of these malignant tumors, but the invasion after 8 weeks was neither deep nor extensive. Metastasis to regional lymph nodes was not found.

Sixteen-week experimental period. Group D—control animals: The epithelium of the buccal pouches was regular with a well-defined stratum corneum. The underlying connective tissue was free of inflammatory infiltration.

Group E—DMBA-painted animals: All animals in this group presented evidence of cellular atypia. Eight animals presented large invasive epidermoid carcinomas. The carcinomas were well differentiated, with varying amounts of keratin being formed with the neoplasms. Bizarre mitoses and bizarre cell forms were not frequently seen.

Group F—DMBA-painted, cortisone-injected animals: Almost all of the animals in this group presented with invasive epidermoid carcinomas. The carcinomas were larger than those in the Group E animals but were histologically of a similar well-differentiated type. Numerous mitoses were observed, but bizarre mitotic figures were rare. Invasion of underlying connective tissue and muscle was more extensive than in the Group E animals. Metastasis to regional lymph nodes was not observed, although in 2 animals there was direct extension to cervical lymph nodes from the large invading buccal pouch tumor.

Discussion

In hamsters whose buccal pouches were being painted with a carcinogenic chemical, systemically administered cortisone served to augment the neoplastic transformation. The various stages in the development of buccal pouch carcinomas have been well described. In the cortisone-injected animals, all these stages were speeded up so that after 8 weeks, invasive carcinomas were found in over 50% of this group, while in the uninjected group, only 1 invasive carcinoma had developed, but many of the animals demonstrated either dysplasia or carcinoma in situ (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>HISTOLOGIC PATTERN OF HAMSTER BUCCAL POUCH PRESENTED BY NUMBERS OF ANIMALS IN EACH OF THE EXPERIMENTAL GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major histologic pattern</td>
<td>EXPERIMENTAL GROUPS (15 ANIMALS IN EACH GROUP)</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Normal</td>
<td>14</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>1</td>
</tr>
<tr>
<td>Hyperkeratosis and dysplasia</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>0</td>
</tr>
<tr>
<td>Invasive epidermoid carcinoma</td>
<td>0</td>
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</table>
After 16 weeks most of the animals presented gross buccal pouch tumors. However, while the cortisone-free group demonstrated well demarcated carcinomas of moderate size and minimal invasion of underlying tissues, the cortisone-injected group demonstrated large, extensive carcinomas with surface necrosis and deep invasion of underlying connective tissue and muscle. The underlying and contiguous tissues were infiltrated and fixed so that in most cases the buccal pouches could not be everted. It was of interest that while these tumors were larger and more extensive, the cellular pattern was similar in both groups and presented a well-differentiated pattern.

Several explanations for the action of cortisone in augmenting carcinogenesis may be offered. The cortisone may behave as a co-carcinogenic influence (3, 7) so that cellular transformation from normal to dysplastic occurs more rapidly. The cortisone, by its antianabolic action on connective tissue, may permit deeper penetration of carcinoma into the underlying corium, through a loss of connective tissue density. Since cortisone depresses antibody formation, it may permit more extensive growth and penetration of the carcinomas by preventing the development of antibody-like substances which would act against the tumor. The function of depressed immunologic reactivity during carcinogenesis has recently received renewed interest (10), based upon the widely accepted concept that neoplasms possess specific antigens capable of eliciting an immune reaction.

Toolan (19) has found that human tumors could be successfully implanted into cortisone-injected animals, while none of the human tumor implants survived into normal controls. Furthermore, it was found that tumors implanted into cortisone-injected and irradiated animals were uniformly larger and more vigorous than those implanted into irradiated animals. This work offers the suggestion that cortisone acts in a manner similar to radiation, in breaking down the organism’s resistance to the growth of a neoplastic lesion, possibly through the inhibition of normal immune mechanisms.

The absence of metastases in our animals is of interest, since it has been shown by Pomeroy (9) that cortisone will induce metastases of transplanted mouse tumors. Baserga and Shubik (2) have found that cortisone favored the metastatic spread of experimental tumors. The fact that the buccal pouch tumors were histologically of the well-differentiated variety may explain the absence of metastatic spread in our experimental groups.

References


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*Cancer Res* 1966;26:2461-2463.

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