Effects of a Chemical Carcinogen on the Submaxillary Gland of Albino Rats Treated with Isoproterenol

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Summary

Ninety 3-month-old male and female albino rats were divided into 6 equal groups. Three groups of animals received daily i.p. injections (20 mg) of isoproterenol for a 2-week period. Pellets of powdered 9,10-dimethyl-1,2-benzanthracene (DMBA) were implanted into the right submaxillary salivary glands of the experimental animals 2 weeks prior to, simultaneous with, and 2 weeks after commencement of isoproterenol injections. The remaining groups of animals served as controls.

The isoproterenol control animals revealed submaxillary gland changes consisting of hypertrophy and hyperplasia of acinar cells and a decrease in the number of ducts. The submaxillary glands of the DMBA-implanted control animals underwent a series of alterations with the formation of well-advanced epidermoid carcinomas at the termination of the experiment. Animals treated with isoproterenol and implanted with DMBA showed a delay in the sequence of alterations and a decrease in the number and size of malignant neoplasms. It is suggested that the salivary gland neoplasms do not arise from acinar tissue, but rather from the ductal elements or from ductlike tissue.

Introduction

It has been shown that daily i.p. injections of isoproterenol will induce notable enlargement of rat salivary glands, consisting of both hypertrophy and hyperplasia of the acinar elements of the gland tissue (6, 7). In contrast, the salivary gland duct system of the isoproterenol-treated animals undergoes degenerative alterations with a subsequent decrease in the number of ducts (4). The induction of salivary gland neoplasms in laboratory animals by the introduction of chemical carcinogens has been well documented by numerous investigators (5, 8, 10). Malignant tumors have been found to develop as early as the 8th week following implantation of chemical and consistently after the 12th week. Recently, it has been demonstrated that submaxillary glands of rats undergo a sequence of changes in response to the implantation of pellets of DMBA (3). The initial phase of the glandular response to DMBA consists of necrosis and degeneration of tissue surrounding the carcinogen. Following this, there is a proliferation of connective tissue around the pellet of DMBA and the formation of numerous ductlike and cystic structures which appear to arise from the transition of ductal epithelium.

Subsequently, the ductal columnar epithelium lining the cystic structures undergoes metaplasia to that of stratified squamous type. Finally, there is a malignant neoplastic alteration of the metaplastic cystic epithelium to epidermoid carcinoma.

It was felt that salivary glands modified by isoproterenol before, simultaneous with, and following implantation with pellets of 9,10-dimethyl-1,2-benzanthracene (DMBA) could be more reactive to the carcinogen and that the induction time, incidence, and type of salivary gland neoplasms may be altered. A study of this type would present further information on the interaction of systemic influences and local exciting factors in experimental carcinogenesis.

Materials and Methods

Ninety male and female albino rats, 3 months of age and weighing approximately 250 gm, were divided into 6 equal groups as follows: Group A, pellets of DMBA were implanted 2 weeks prior to the commencement of isoproterenol injections; Group B, pellets of DMBA were implanted simultaneous with the commencement of isoproterenol injections; Group C, pellets of DMBA were implanted 2 weeks after commencement of isoproterenol injections; Group D, isoproterenol controls; Group E, DMBA controls; Group F, untreated controls.

The pellets of pure DMBA (9,10-dimethyl-1,2-benzanthracene) weighed approximately 5 mg and were implanted into the surgically exposed right submaxillary salivary gland by a previously described method (2). The isoproterenol injections were administered i.p. and were given daily for a 2-week period. Each injection consisted of 20 mg of isoproterenol dissolved in 0.2 ml of distilled water. Five animals from each group were sacrificed with diethyl ether at 4 weeks, 8 weeks, and 20 weeks following implantation with pellets of DMBA. (Group D animals were sacrificed at the same intervals after the commencement of isoproterenol injections.)

Both right and left glandular masses were removed, the tissue was bisected, and the remaining portion of the pellets of carcinogen was removed. The tissue was fixed in formalin for 48 hr, embedded in paraffin, and stained with hematoxylin-eosin.

Results

GROSS OBSERVATIONS. In the isoproterenol control animals (Group D) marked and rapid enlargement of the salivary glands was evident during the 2-week injection period. However, this glandular enlargement could not be visualized or palpated 3 weeks after cessation of injections. In the DMBA control ani-
Maligns (Group E) small firm nodules could be palpated in the right submaxillary gland as early as 1 week following implantation. These nodules slowly enlarged, and at the termination of the experiment some animals developed large ulcerating masses in the neck. The isoproterenol-DMBA groups of animals (Groups A–C) revealed glandular enlargement throughout the 20-week experimental period, characterized by the rapid enlargement of salivary gland tissue apparently due to the isoproterenol injections and the slow progressive enlargement representing tissue reaction to the DMBA. Confirmation of this awaited histologic study.

Microscopic observations. At the 4-week experimental period the submaxillary glands of the isoproterenol control animals (Group D) revealed a marked enlargement of acinar cells and a decrease in the number of ducts. The acinar cells were characterized by a coarsely granular eosinophilic cytoplasm. The nuclei were enlarged, hyperchromatic, and located at the basal portion of the cell. Secretory ducts which remained had undergone regressive changes as evidenced by disruption of cell architecture, degeneration, and necrosis (Figs. 1, 2). There were areas of fibrous connective tissue replacement of glandular tissue and a persistence of some of the striated ducts. The animals sacrificed at 8 weeks presented glandular tissue with a normal configuration, residual acinar enlargement, and areas of fibrosis. At the 20-week sacrifice interval, except for areas of fibrosis, the glandular tissue had returned to normal.

The DMBA control animals (Group E) sacrificed at 4 weeks showed a mass of dense hyaline-like connective tissue, in various stages of degeneration, adjacent to the space occupied by the carcinogen. Proliferation of ductlike and cystic structures was noted in the hyaline-like connective tissue (Fig. 3). At the 8-week sacrifice interval the cystic structures had enlarged and the cuboidal lining epithelium was altered to that of the stratified squamous type (Fig. 5). All DMBA-implanted animals sacrificed at 20 weeks revealed well-advanced epidermoid carcinomas arising from the epithelium of the cyst wall (Fig. 7).

In the Group A animals (isoproterenol injections given 2 weeks after the implantation of DMBA) enlarged acinar cells were noted at the 4-week sacrifice interval, but the tissue reaction to the DMBA was as advanced as that of the DMBA control group. At the subsequent periods of sacrifice, the animals of Group A were histologically indistinguishable from those of the DMBA control (Group E).

In contrast, the animals in Groups B and C showed a decrease in the formation of ductlike and cystic structures at the 4-week sacrifice interval (Fig. 4). It appeared that the decrease in the number of ductlike and cystic structures was directly related to the isoproterenol-induced decrease in the number of salivary ducts. In Group B and C animals sacrificed at 8 weeks the acinar cells had for the most part returned to normal, but there were areas of residual acinar enlargement. There was a gradual reappearance of ductal elements, but the tissue reaction to the carcinogen was not as advanced as that of the DMBA controls. Proliferation of the ductlike structures was also evident, but relatively few areas of cyst formation and metaplasia of ductal epithelium were noted (Fig. 6). However, the animals sacrificed at 20 weeks revealed epidermoid cyst formation with early malignant alterations in the cystic epithelium (Fig. 8). Although malignant tumors developed in the Group B and C animals at 20 weeks, they were smaller and less advanced than the tumors in the DMBA control group, apparently owing to the delay in the sequence of tumor formation (Table 1).

Discussion

The observations in this study are of considerable interest in their elucidation of various aspects of the complex problem of salivary gland carcinogenesis. There has been a great deal of discussion and controversy as to the specific tissue elements affected by the experimental carcinogenic agents (1, 9, 10). This study would appear to suggest that the ductal elements of the gland are primarily affected. In the isoproterenol-injected animals, where there is a degeneration, necrosis, and reduction in ductal tissue, there is a reduction in carcinogenic activity. Less tumors are produced, and the over-all sequence of neoplastic transformation is retarded. The acinar elements of the gland undergo hypertrophy and hyperplasia, rather than degeneration. It was thought that this tissue, undergoing hyperplasia, might

### Table 1

**Dominant Histologic Pattern Induced by DMBA**

<table>
<thead>
<tr>
<th></th>
<th>4 Wk.</th>
<th>8 Wk.</th>
<th>20 Wk.</th>
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<tbody>
<tr>
<td></td>
<td>Group</td>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Proliferation of ductlike structures</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Early cyst formation</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cyst and metaplasia</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early carcinoma in cyst</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Advanced carcinoma with invasion</td>
<td>0</td>
<td>0</td>
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* Group A, pellets of DMBA implanted 2 weeks prior to the commencement of isoproterenol injection; Group B, pellets of DMBA implanted simultaneously with the commencement of isoproterenol injection; Group C, pellets of DMBA implanted 2 weeks after commencement of isoproterenol injection; Group E, DMBA controls.*
Effects of Carcinogen on Submaxillary Gland after Isoproterenol Display an increased susceptibility to carcinogenic influences. Since this did not occur, it could be suggested that the salivary gland neoplasms do not arise from acinar elements, but rather from ductal elements or from ductlike tissue replacing necrotic acinar tissue. Neoplastic transformation is usually seen in the walls of the cystic structures that develop from ducts or ductlike tissue.

It is of interest that a systemic influence, such as a sympathomimetic drug, can act to retard carcinogenesis in an organ affected by this systemic influence. Our microscopic observations serve to clarify this finding and to explain the carcinogenic retardation at a histologic level.

No differences were noted in the types of tumors developing in response to the DMBA in the various experimental groups. The only differences apparent were a delay in the sequence of salivary gland carcinogenesis and a reduction in the number and size of tumors produced.

References

Fig. 1. Normal submaxillary gland demonstrating secretory tubules and acinar elements. H & E, × 180.
Fig. 2. Submaxillary gland of animal treated with isoproterenol. Note enlargement of acinar cells and decrease in number of ductal elements. The ducts which remain are undergoing degenerative alterations. H & E, × 180.
Fig. 3. Submaxillary gland of 4-week DMBA control animals showing metaplasia of epithelium and cyst formation. H & E, × 180.
Fig. 4. Submaxillary gland of 4-week DMBA-isoproterenol-treated animals. Proliferation of ductlike structures is noted as well as enlarged acinar cells. There is no cyst formation. H & E, × 180.
Fig. 5. Submaxillary gland of 8-week DMBA control animal showing portion of the lining of a large epidermoid cyst and epithelial proliferation. Note normal submaxillary gland tissue at the lower portion of the photomicrograph. H & E, × 180.
Fig. 6. Submaxillary gland of 8-week DMBA-isoproterenol-treated animal revealing proliferation of ductlike structures and early cyst formation. Residual enlargement is noted in the acinar cells. H & E, × 180.
Fig. 7. Submaxillary gland of 20-week DMBA control animal. Well-advanced epidermoid carcinoma is seen arising from the wall of an epidermoid cyst. H & E, × 180.
Fig. 8. Submaxillary gland of a 20-week DMBA-isoproterenol-treated animal revealing cyst wall with epithelial proliferation and early malignant transformations. H & E, × 180.
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