Auditory Canal Structures in Rats as Altered by Aging and by the Administration of Tris(p-aminophenyl)carbonium Pamoate

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Summary

This study was concerned with changes in the auditory canal and its associated structures in rats as affected by aging and by oral administration of tris(p-aminophenyl)carbonium pamoate (TACP). Multiple sections of tissue blocks from the auditory canal area were reviewed in control and treated animals of both sexes, and of varying ages up to 18 months. Tumors were found in the auditory canal gland of both treated and control animals, but with greater incidence and shorter latent period in the treated animals. The incidence of tumor formation was higher in female than in male rats. Further, tumors were found in the sebaceous glands of the ear canal, but in the treated animals only. Cystic glandular changes related to aging appeared to contribute to tumor formation and development.

Introduction

During a preliminary study in albino rats fed a diet containing tris(p-aminophenyl)carbonium pamoate (TACP), there was an increased incidence of tumors which involved skin sebaceous glands, mammary glands, intestine, subcutaneous tissue, and auditory canal gland (4). We were particularly interested in the pathogenesis of those tumors in the area of the auditory canal gland because of conflicting accounts in the literature concerning their nature. Accordingly, from these animals, the ear canal and associated structures were specially sectioned for further study. We also considered that a review of the changes of aging might be pertinent since a few spontaneous tumors of the auditory canal gland have been described (9, 11). The present study describes these additional, more extended morphologic findings.

Materials and Methods

TACP is chemically described as the tris(p-aminophenyl)carbonium salt with one-half formula weight 4,4'-methylenebis(3-hydroxy-2-naphthoic acid). The chemical characteristics of TACP and the experimental design of the study have been described in detail (4).

Three groups of Holtzman rats were used; 2 groups were given separate lots of drug incorporated in the diet at a concentration of 0.1-0.2%, and the 3rd group served as a control (standard ration). Planned sacrifices were performed at 6 months (females only), and at 18 months (males and females). Morphologic observations are grouped on a quarterly (3 month) basis for the full 6 quarters, and include data from the animals dead or sacrificed during these time intervals. The auditory canal and its associated structures were dissected from control and treated rats by cutting a rectangular piece of tissue of approximately 10 × 20 mm from the ear region which included the auditory canal gland and its associated structures, the tympanum and middle ear, the parotid gland, and cheek muscle. This block was fixed in 10% buffered neutral formalin, decalcified in Ver- sense, sectioned in paraffin 6 μ thick, and stained with hematoxylin and eosin. Multiple slides (about 15) were made of each block in order to insure sections of each of the structures; however, since not all blocks yielded wholly satisfactory sections, our final study was confined to those which fulfilled our requirements (132 treated, 121 control animals).

In studying the changes, the principal sites of concern included the anterior, (a) and posterior (b) sebaceous glands of the auditory canal, the tympanum and middle ear (c), and the auditory canal gland (d). The anatomical relationship of these sites is shown in Fig. 1.

In our microscopic study, we reviewed the epithelial structures, their size, and cytologic and structural changes, particularly as related to tumor formation; in the middle ear we reviewed the evidences of inflammation and the changes in the membrana tympani.

Results

We have combined the results from the 2 drug-treated groups since the findings were sufficiently similar in both. These results, compared to the controls, are summarized in tabular form in Table 1.

ANTERIOR AND POSTERIOR CANAL SEBACEOUS GLANDS. The sebaceous glands of the anterior and posterior walls of the auditory canal in the young rat consisted of numerous simple sebaceous glands lying just beneath the squamous epithelial lining of the canal, proximal to the auditory canal gland. The glands of the anterior wall were composed of larger, more lobulated sebaceous glands arranged in aggregates (Fig. 2a), while those of the posterior wall were composed of smaller, solitary follicular cells superficially oriented (Fig. 3a). The glands of the posterior wall extended to, and overlapped, the tympanic membrane.

With increasing age, there was a general increase in size, prominence, and apparent cystic dilation of the glands. These changes were more exaggerated in glands of the anterior wall (Figs. 2b, 2c) than those of the posterior wall (Fig. 3b), and occurred equally in male and female animals. These changes...
TABLE 1
SUMMARY OF CHANGES IN THE AUDITORY CANAL GLAND AND AUDITORY CANAL SEBACEOUS GLANDS OF RATS OVER AN 18-MONTH PERIOD

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Sex</th>
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<th>No. Examined</th>
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were generally more prominent in treated animals than in untreated animals. Significantly, there was adenoma formation of the glands of the anterior wall, but not of the posterior wall, in 10 (7.5%) of the treated animals (4 males, 6 females). The smallest lesions appeared as cystic adenomas, small solid adenomatous nodules, or larger adenomatous tumors composed of sebaceous cells lying in the anterior wall just beneath the squamous cell lining of the auditory canal (Figs. 4a-c). Later there was organized enlargement of the cystic or nodular adenomas with protrusion into the canal, and with the appearance of small, but well-developed nodules projecting from the anterior wall with extension into the canal (Fig. 4d). These tumors had squamous metaplasia and prominent keratinization. They occurred at an average age of 286 days (death or sacrifice). Six of these tumors occurred in animals which also had tumors of the auditory canal gland.

MEMBRANA TYPANII. The tympanum in the young rat consisted of a thin fibrous membrane stretched tightly across the auditory canal. It was bounded on its external surface by a thin squamous cell layer which was continuous with the lining of the auditory canal. Sebaceous glands of the posterior wall overlapped its attachment.

With increasing age there was an increased thickness of the membrane and, in some cases, squamous cell hyperplasia of the canal surface (Fig. 5a). Of the control animals ranging from 7 to 549 days of age, 103 (85%) had otitis of varying degree, and of the treated animals ranging from 8 to 549 days of age, 115 (87%) had otitis media. Males of both groups had a greater incidence than females. The otitis ranged from a mild inflammation to massive suppuration of the middle ear, with accumulation of purulent and necrotic tissue in the middle ear cavity. In those animals of the control group with otitis, 16 (13%) had perforation of the tympanic membrane with extrusion of debris into the auditory canal (Fig. 5b), whereas 11 (9%) of the treated...
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group had perforation of the membrana tympani. The incidence and severity of otitis media and the incidence of perforation were not related to increasing age of the animals in either group.

AUDITORY CANAL GLAND. The normal auditory canal gland has been described in detail (13, 14). It is located anterior to the ear, medial to the temporal bone, and is projected from the external auditory canal, and lying between it and the lower jaw. The gland consists of 4 lobules, each of which has a minor (intralobular) duct, which eventually form a main secretory duct. This opens into the anterior-inferior wall of the external auditory canal near the tympanic membrane by passing through the cartilaginous and bony plates of the auditory canal.

Grossly, in the young rat the auditory canal gland consisted of a small, triangular shaped pinkish yellow structure, and microscopically was composed of cells clustered into lobules. Portions of the minor ducts were evident within the lobules. The intralobular ducts united to form a major secretory duct lined by stratified squamous epithelium, which emerged finally into the auditory canal. The lobules were composed of compact, rounded, pale staining sebaceous cells with prominent cell membranes and centrally with varying degrees of cytoplasmic and nuclear degeneration. These sebaceous cells rested on several rows of flattened basophilic basal cells.

The major ducts of the auditory canal gland underwent no significant change with aging, nor was there any evidence of occlusion. There were, however, occasional instances of superficial squamous metaplasia. With increasing age in both treated and untreated animals, there was a progressive increase in severity and incidence of dilatation of the lumen of the minor ducts within the lobules, producing a slight cystic appearance (Fig. 6a). In most of the older animals, there was frank cyst formation which, in some, was quite prominent (Fig. 6b). This was perhaps slightly more prominent in male than in female animals. In many cases, there was an associated inflammation and granuloma formation, and the cyst lumina sometimes contained debris. In many instances, there was disruption of the sebaceous cells emptying into the minor ducts, with sloughed cells and/or cellular debris within the dilated spaces. In no case was there obstruction of any of the minor ducts. Additionally, glands increased in size, the extent and incidence of which was a function of aging. In occasional instances there was a generalized hypertrophy of the gland without cystic dilatation, but in most cases cystic change and increased gland size occurred together.

In 3 (2.5%) untreated rats (2 females, 1 male) sacrificed at Days 317, 463, and 485, respectively, there were small adenomatous nodules in the auditory canal gland (Fig. 7) which had arisen in deeper portions of the lobules in cystic glands. In 35 (26.5%) treated animals, there were 43 similar tumors. These were classed as either cystic adenomas (14 occurrences) or papillary epithelial tumors (29 occurrences). The adenomas occurred predominantly in females, as did the papillary tumors. The latter were first observed grossly as early as Day 189; the average age of the animals at death or sacrifice was 311 days. Over-all tumor incidence in treated animals was 28/57 (49.1%) for females, and 7/75 (9.3%) for males.

In most of the tumors there were several cell arrangements which fitted the following description. The earliest tumors observed in the auditory canal gland consisted of small adenomatous nodules within dilated cystic portions of a lobule, usually at the base of the gland (Fig. 8); from this there were varying stages to massive, solid papillary epithelial tumors (Fig. 9). In some cases, there was enlargement of the initial nodule, with extensive cyst formation (Fig. 10a). In others, cyst formation was not prominent (Fig. 9). Many were intermediate between these types. Some of the tumors had residual sebaceous cells with squamous metaplasia and mild keratin formation; others were more cystic, with additional squamous metaplasia and keratinization, resulting in epithelial pearl formation (Fig. 10b). Growth of the tumors was striking. Many tumors enlarged beyond the dilated minor ducts of the gland to protrude through the major ducts into the ear canal. In some cases this superficially suggested a ductal origin (Fig. 10c). Others enlarged in several directions, at times eroding through the bony plate of the ear canal and filling the canal or invading the middle ear cavity. Neither stasis within the gland nor obstruction of the duct of the gland contributed to the incidence of tumor development. In 3 animals there were 2 quite distinct tumors in the same auditory canal gland, and these were in all respects similar to the variants described above. In 5 other animals there were bilateral tumors. Four of these animals had a papillary epithelial tumor in each gland, and 1 had a papillary epithelial tumor on 1 side, and a cystic adenoma on the other side. One animal had pulmonary metastases from a squamous papillary tumor.

Discussion

The auditory canal gland and its associated structures are altered with increasing age. The changes in the auditory canal gland can be briefly summarized as cystic dilatation of the lobules, sometimes with inflammation, or enlargement of the gland. The sebaceous glands of the canal wall were characterized by increased prominence of the glands and cystic dilatation. These changes were more marked in the anterior than in the posterior wall. The membrana tympani became thickened and sometimes hyperplastic. This was possibly related more to prevalent otitis than to aging. In addition, tumors were observed in the auditory canal gland in both treated and untreated animals, and in the anterior canal sebaceous glands in TACP-treated animals. Thus, either site of origin must be considered possible since even careful dissection and multiple sections may not allow distinction owing to the close histologic similarity of tumors from these areas. Further, multiple tumors may occur in the same gland, or the tumors may be bilateral.

Spontaneous tumor development in the auditory canal gland of the rat has been described in several recent reports. In 1 study (11), 3/105 untreated Sprague-Dawley rats developed sebaceous gland carcinomas at an average age of about 800 days. In another report (9), "3 squamous cell carcinomas of the ear canal occurred in OM rats and 2 in M520 rats, all over 21 months of age," in addition to "1 squamous papilloma of the ceruminous gland in a 24 month old ACI male." In the present study, we observed cystic adenomas of the auditory canal gland in 3/121 untreated rats with an average age of about 420 days. Each of these tumors arose as a small, adenomatous nodule within deeper cystic portions of the gland.

Tumors of the auditory canal gland have been reported
following administration of urethan (11), certain aromatic amines: 2-acetylaminofluorene (1, 5, 8), benzidine (10), 4-aminodiphenyl (12), 4-amino stilbene (3), and 9:10-dimethyl-1,2-benzanthracene (2), as well as an azo dye, 3-methoxy-4-aminoa zobenzene (6). The tumors encountered have invariably been described as sebaceous or keratinizing squamous carcinomas. Tumor incidence with these agents has ranged from 17 to 57%, with an average latent period of 160-430 days, and with females more susceptible than males. In this experiment, tumors of the auditory canal gland were induced with a total incidence of 26.5%, in both sexes) with a triphenylmethane dye, TACP. The tumors were classified either as cystic adenomas or papillary epithelial tumors. The adenomatous tumors were increased in incidence, and had a shorter latent period in the treated animals compared to the controls.

There has been some uncertainty concerning the origin and development of these tumors, and they have been variously described as arising from the region of the auditory canal, the external acoustic duct, and of the side of the head. Cox et al. (1) believed the origin of the tumors to be most likely some accessory structure rather than the lining of the auditory canal. Spitz et al. (10) were unable to trace the origin of the tumors due to the overshadowing of the tumor elements by keratinization. Skoryna et al. (8) thought the tumors had their origin in the auditory canal gland, arising in cystic lobules as a result of stasis of secretion, and consequent to otitis media. To the contrary, Laws et al. (5) reported the absence of generalized hyperplasia, and stated that neither cystic changes in the ducts nor otitis media were related to tumor development. They concluded that the tumor had a glandular origin involving the entire lobule of the auditory canal gland. Morris et al. (7), in histologically dissimilar tumors, believed the ciliated mucoid goblet cells lining the middle ear and eustachian tube to be the cell of origin of head tumors. In our study, review of multiple sections of the auditory canal gland would support the view that most tumors do indeed originate in the auditory canal gland. These were seen initially as small adenomatous nodules within cystic, dilated portions of a lobule within the depths of the gland. Neither stasis, inflammation, nor obstruction were observed regularly in association with the tumors. The presence of otitis had no appreciable influence on tumor development. We suggest that lobular cystic dilation in the gland related to aging, contributes to tumor formation and development. These changes were further accentuated by TACP administration. Additional support for this view is found in the observation that, when tumors arose in the sebaceous glands of the anterior canal wall, they apparently had origin in cystic glands, common in older rats.

Acknowledgments

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References

Fig. 5. Membrana tympani. a, Marked squamous hyperplasia related to aging and otitis. b, Perforation associated with otitis. × 100.

Fig. 6. Auditory canal gland. a, Cystic changes grade I related to aging. b, Cystic changes grade III related to aging. × 50.

Fig. 7. Cystic adenoma of auditory canal gland in an untreated rat. × 50.

Fig. 8. Early adenomatous nodule of auditory canal gland in TACP-treated rat. × 100.

Fig. 9. Papillary epithelial tumor of auditory canal gland in TACP-treated rat. × 100.

Fig. 10. Histologic variations in tumors of TACP-treated rats. a, Cyst formation in adenomatous tumor. × 50. b, Cystic squamous metaplasia and keratinization in a tumor. × 100. c, Protrusion of tumor elements from major duct (top right of figure) of gland. In other sections the continuity was established. × 100.
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