Induction of Neoplasms in Hamster Tracheal Grafts with 3-Methylcholanthrene-coated Lycra Fibers

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ABSTRACT

Synthetic Lycra spandex fibers were coated with 3-methylcholanthrene (3MC) and inserted into the lumina of 104 tracheas removed from young adult inbred Syrian hamsters. Tumors appeared 3 to 21 months after implantation of these tracheal grafts in syngeneic animals. 3MC in amounts ranging from 9 to 75 μg/fiber induced a diversity of neoplasms in tracheal epithelium, the majority of which were classified histologically as carcinomas. Tumors failed to develop when smaller amounts of 3MC were used and in control tracheal grafts containing fibers without carcinogen. The use of tracer quantities of [14C]-3MC allowed quantitation of the carcinogen dosage to the mucosa before and at intervals after grafting. These studies demonstrate the dosage-dependent sensitivity of the hamster tracheal epithelium to relatively small quantities of 3MC. The direct application of carcinogens coated on synthetic fibers provides a useful tool for assaying water-insoluble carcinogens.

INTRODUCTION

Heterotopically transplanted rat tracheas have been used in several laboratories for the evaluation of the carcinogenicity of PCH (2, 3, 5, 6, 12, 13), nitrosamines (5, 17), and metal carbonyls (6). Agar (6), gelatin (5), and beeswax (2, 3, 12, 13) are used as vehicles for the chemicals, presumably allowing topical application of the carcinogen to the mucosa over an extended period. Squamous cell carcinomas and adenocarcinomas have been induced reproducibly in the rat with this technique (2, 3, 5, 6, 12).

The approach developed by Griesemer et al. (2) has been modified in our laboratory in an effort to improve the quantitation of carcinogen dosage to the tracheal epithelium. Synthetic fibers coated with 3MC were inserted into excised hamster tracheas that were subsequently grafted on syngeneic animals. A diversity of neoplasms was induced with dosages of 3MC several- to 100-fold lower than the amounts of PCH used by others (3, 5, 6, 12). 3MC labeled with [14C] was used to quantitate carcinogen release into the lumen of the tracheal graft.

MATERIALS AND METHODS

Fibers. In preliminary studies a variety of synthetic fibers was evaluated to determine (a) the uptake of [14C]-3MC after immersion of the fiber in an acetone solution containing the carcinogen and (b) the retention of the PCH after incubation in an aqueous medium for as long as 8 weeks. Since Lycra spandex appeared to allow a steady release of the carcinogen, these fibers were used in the experiments described below. Over an 8-week period 20 to 25% of the 3MC initially bound to the fiber is delivered to the surrounding tissues. Lycra is comprised of numerous filamentous subunits (Fig. 1); this increases the surface area for adsorption of PCH.

Type 126 Lycra spandex fibers (560 denier, obtained from H. P. Bodenstab, Textile Fibers Department, E. I. du Pont de Nemours & Co., Wilmington, Del.) were cut to a length of 8 mm, washed in acetone, sterilized at 120° for 20 min, and placed in sterile glass vials. 3MC (Aldrich Chemical Co., Milwaukee, Wis.) was diluted in cold (4°) analytical-grade acetone at concentrations of 30, 15, 10, 3, 1, and 0.3 mg/ml. Five ml of each solution were added to individual vials; the vials then were stored with loose tops at 4° for 1 week to permit evaporation of the solvent. After 1 week residual acetone was removed under nitrogen.

In a parallel experiment [14C]-3MC was mixed with unlabeled 3MC at a ratio of 1:10 for assay of the amount bound to individual fibers. The fibers were coated as described above, and the 3MC on individual fibers was extracted and counted in toluene. Standard curves were made from known amounts of [14C]-3MC (New England Nuclear, Boston, Mass.). The least-squares fit of the logs technique was used to analyze data. The mean amount of carcinogen per 8-mm fiber, determined by assaying 10 fibers from each vial was 75 ± 2.4 (S.E.), 32 ± 0.7, 17 ± 1.5, 9 ± 0.9, 3 ± 0.3, and 1 ± 0.03 μg, respectively. After suspension in 30 mg of 3MC per ml of acetone (a supersaturated solution), the maximum amount of 3MC adsorbed was approximately 9.5 μg/mm of Lycra fiber.

Experimental Design. Four- to 6-week-old female hamsters of the 15.16 Hamburger strain (TELACO, Bar Harbor, Maine) were administered tetracycline-HCl for 5 days (9) and then killed with Nembutal. The intact trachea was excised by sharp dissection from the larynx to the bifurcation and immersed in Hanks' balanced salt solution, and the extraneous tissues were removed by dissection. A total of 104 tracheal grafts was used. Fibers coated with 3MC or acetone-washed control fibers were inserted into the lumen of excised tracheas that were then closed at each end with Ethicon silk sutures.

Twenty-two syngeneic weanling female recipients were anesthetized with Nembutal; tracheal grafts were implanted s.c. into the back of each animal. In Experiment 1 individual animals were given implants of 3 tracheal grafts, each containing a [14C]-3MC-coated fiber. Recipient hamsters in Experiment 2 were given implants of 4 grafts, each contain-
ing a fiber coated with unlabeled 3MC. A graft with an acetone-washed fiber also was implanted into each animal. Skin incisions were closed with Autoclips.

Animals were examined at 3-week intervals for the detection of tumors, and the grafts were removed when masses approximately 5 mm in diameter were palpated. At 8 months 2 animals lacking palpable masses were killed, and the grafts were excised for histological study. Two hamsters with invasive tumors died at 7 and 9 months. The fibers removed from these animals were assayed for 14C to estimate the approximate amount of 3MC released into the graft. Because the majority of tumors in Experiment 1 developed at 5 to 10 months after implantation, Experiment 2 was terminated at 12 months. Animals were autopsied routinely after removal of the tracheal grafts.

Histopathology. After the removal of Lycra fibers from the grafts, tissues were fixed in Bouin's solution overnight and then transferred to buffered formalin (pH 7). Tracheas were embedded on end in paraffin, and serial 5-µm cross-sections were prepared. One of each 25 serial sections was examined microscopically after staining with hematoxylin and eosin for determination of the site of origin of the neoplasm. Tissues were stained selectively for mucin (7) and reticulin fibers (18) and by the Masson's trichrome (8) and eosin for determination of the site of origin of the neoplasm. Tissues were stained selectively for mucin (7) and reticulin fibers (18) and by the Masson's trichrome (8) and periodic acid-Schiff (4) techniques. In addition, fragments of tumor were fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Ultrathin tissue sections were stained with uranyl acetate and lead acetate and examined with a Philips 300 electron microscope.

Transplantation of Tumors. After the removal of tumors from grafted recipient animals, fragments (approximately 2 cu mm) were transplanted into syngeneic female hamsters. Alternatively, tumor tissue was trypsinnized into cell suspensions, and monolayers of cultured cells were selectively transplanted as cell pellets (approximately 1 to 3 × 10⁶ cells/animal) into syngeneic weanling hamsters.

RESULTS

Tracheal Grafts. Tracheal grafts containing acetone-treated Lycra fibers exhibited a well-differentiated mucociliary epithelium 12 months after implantation. The lumen usually was filled with material that stained by the periodic acid-Schiff technique, presumably mucin and serous fluids. Similar features were observed in many of the carcinogen-treated grafts that failed to develop tumors. However, focal areas of squamous metaplasia and a low, cuboidal epithelium frequently were found (Fig. 2).

Malignant tumors appeared in tracheal grafts containing Lycra fibers coated with ≥9 µg of 3MC. The proportion of grafts developing neoplasms increased with the dosage of carcinogen and the duration of exposure (Table 1). The types of cancers induced by 3MC are summarized in Table 2 and illustrated in Figs. 3 to 7. The majority were carcinomas (either invasive or noninvasive), although at the highest dosage of 3MC (75 µg/fiber) 3 of the 12 tumors were fibrosarcomas.

Histological step sections of grafts were studied to define the site of origin of the neoplasm and the morphological features of the tumor. In all but 3 of the 22 grafts developing carcinomas, highly atypical and malignant epithelial cells were found in the tracheal mucosa adjacent to sites of invasive tumor. In several of the well-differentiated epidermoid carcinomas, there was a progressive dedifferentiation of epithelial cells associated with deep invasion of the submucosal and peritracheal tissue.

Tumors developed in hamsters implanted with fragments of the primary carcinomas and suspensions of tumor cells grown in vitro. The latency periods until the time of appearance of palpable neoplasms varied from 2 to 22 weeks after implantation. These lesions often lacked the differentiated

<p>| Table 1 |
| Tumor induction by 3MC-coated Lycra fibers in tracheal grafts |</p>
<table>
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<th>Dosage (µg/8-mm fiber)</th>
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<th>3-5 mos.</th>
<th>6-8 mos.</th>
<th>9-11 mos.</th>
<th>≥12 mos.</th>
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<p>| Table 2 |
| Histological class of tumors induced in tracheal grafts by 3MC-coated Lycra fibers |</p>
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<th>3-5 mos.</th>
<th>6-8 mos.</th>
<th>9-11 mos.</th>
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IC, carcinoma, poorly differentiated; SmC, small cell carcinoma; SqC, squamous cell carcinoma; NC, noninvasive carcinoma; P, papilloma; M, mixed carcinomatous and sarcomatous elements; F, fibrosarcoma; AC, adenocarcinoma.

a Nonradioactive 3MC was used. The 3MC dosage was determined by assaying [14C]-3MC fibers prepared in an identical fashion.

a ND, not done.

a C, carcinoma, poorly differentiated; SmC, small cell carcinoma; SqC, squamous cell carcinoma; NC, noninvasive carcinoma; P, papilloma; M, mixed carcinomatous and sarcomatous elements; F, fibrosarcoma; AC, adenocarcinoma.

a A poorly differentiated neoplasm with focal well-differentiated elements of squamous cell carcinoma and a mucus-secreting adenocarcinoma. A well-differentiated lesion developed as a recurrence in the primary excision of the primary tumor mass.
features of the primary neoplasm and appeared to be anaplastic carcinomas.

In Experiment 1 approximately 30 cells from 5 anaplastic tumors were studied ultrastructurally. Functional complexes between cells were observed commonly. In cells of 2 neoplasms, intracellular cilia of “9-2” centriolar structure were found (Fig. 8). Virions resembling hamster R-type particles (15) and intramitochondrial virus-like particles were demonstrated in cells from 3 of the 5 cancers (Fig. 9).

Release of 3MC from Lyca Fibers. For determination of the amount of carcinogen released into the lumen preceding the development of tumors, fibers labeled with [14C]-3MC (Experiment 1) were removed from the excised grafts of 2 animals at 8 months (Table 3).

DISCUSSION

The development of tools for the quantitative assessment of respiratory carcinogens poses a challenge for the experimental oncologist. In humans it appears that carcinogen dosages in the respiratory tract are low and exposure times are protracted over periods of years or decades. The studies reported here were undertaken in an effort to develop an assay for substances producing epithelial neoplasms under conditions roughly simulating human exposure. Moreover, we were interested in perfecting a technique to determine whether a continuous carcinogenic stimulus was necessary for tumor induction.

Previous studies with intact animals have shown that carcinomas can be induced in hamsters when PCH are inhaled at concentrations that would not be considered inconsequential. At the highest concentration of 3MC, approximately 0.03 µCi of 14C were released into the graft.

The tumors developing in hamsters often were relatively undifferentiated squamous cell carcinomas. To confirm the epithelial origin of these lesions, we routinely examined serial histological sections of the graft to demonstrate the site of origin of the tumor and changes in the adjacent mucosa. These studies demonstrated preneoplastic and neoplastic changes in the intact respiratory mucosa similar to those reported by others (3, 12).

Previous studies have shown that 40 to 300 µg of either benzo(a)pyrene or dimethylbenzo(a)anthracene are required to produce carcinomas in the grafted rat trachea (3, 12). In our studies the carcinogenic dosage of 3MC was substantially less. It is unclear at present whether the mode of application of the carcinogen or the species used was the determining factor. Regardless, the tracheal graft technique with the hamster is a bioassay with a sensitivity for carcinogens substantially greater than that of previously described models.

Of interest was the diversity of tumors developing in this model system. Although some exhibited the typical features of well-differentiated keratinizing squamous cell carcinomas and adenocarcinomas, a consistent histological pattern related to the dosage of carcinogen was not observed in the relatively small number of tumors examined. However, differentiated elements often were found embedded in a mass of less-well-differentiated neoplastic epithelial cells. We have the impression, based upon the study of serially prepared tissue sections, that a progressive differentiation of the carcinomas occurs as the tumor invades through the muscular wall of the graft and into the adjacent host tissue. Fibrosarcomas developed in 3 grafts exposed to relatively high concentrations of 3MC. It appears that the dosage was sufficient in these cases to transform mesenchymal elements beneath the epithelium. A detailed review of our histological findings in these and other studies reported elsewhere (10) is in press (1).

ACKNOWLEDGMENTS

The excellent technical assistance of Betty Clements, Laurie DiCesare, Brenda Ley, Judith Kessler, Bonnie MacLeod, and Gail Turnbull is appreciated.

REFERENCES

B. T. Mossman and J. E. Craighead


Fig. 1. Scanning electron micrograph of Lycra. The cross-sectional illustration shows the many fine filaments comprising the fiber. Gold palladium, x 75.

Fig. 2. Focal nonkeratinizing squamous metaplasia in a tracheal graft exposed to 1 µg of 3MC for 5 months. H & E, x 2,000.

Fig. 3. A, a poorly differentiated carcinoma originating in a tracheal graft exposed to 9 µg of 3MC for 10 months. The tumor extends from the luminal surface into the adjacent s.c. tissue of the host. H & E, x 20. B, neoplastic epithelial cells from the inset of A. Note invasion into the underlying stroma. S, submucosa. H & E, x 1,600.

Fig. 4. A poorly differentiated carcinoma in a tracheal graft exposed to 9 µg of 3MC for 10 months. Note the polygonal morphology of the tumor cells. H & E, x 175.

Fig. 5. Focal lesion of a squamous cell carcinoma found adjacent to a much larger mass of poorly differentiated carcinoma. This tumor developed in a tracheal graft exposed to 17 µg of 3MC for 5 months. H & E, x 1,200.

Fig. 6. Focal area of well-differentiated adenocarcinoma in the same neoplasm illustrated in Fig. 5. The material within the glandular lumina stained positively by the mucicarmine technique. Mucicarmine, x 300.
Fig. 7. A small cell carcinoma in a graft exposed to 17 μg of 3MC for 4 months. The tumor invaded the submucosa and adjacent tracheal cartilage. H & E, x 400. Inset, cord-like arrangement of cells in the tumor mass. H & E, x 1,400.

Fig. 8. Intracellular cilium with "9-2" centriolar structure. Cilia were observed occasionally in neoplastic cells from poorly differentiated carcinomas. Similar structures are seen in poorly differentiated human bronchial carcinomas (16). Uranyl acetate and lead citrate, x 14,700.

Fig. 9. Virus-like particles found in tumors. Arrow, spoke-shaped core of the virion. A, intracisternal H- or R-type particles (outer diameter 105 to 110 nm). Uranyl acetate and lead citrate, x 27,000. B, intramitochondrial viral particles (outer diameter 88 to 95 nm). Uranyl acetate and lead citrate, x 22,500.
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