Bronchial and Pulmonary Carcinogenesis at Focal Sites in Dogs and Hamsters

John R. Benfield and William G. Hammond
Department of Surgery, Division of Cardiothoracic Surgery, University of California at Davis School of Medicine, Davis, California 95616

Abstract
Models of the sequential process of lung carcinomas have been developed in dogs and hamsters. The bronchial mucosa, or the pulmonary parenchyma, was exposed at selected focal sites to polycyclic aromatic hydrocarbons [most often benzo(a)pyrene or methylcholanthrene]. In hamsters, sustained release implants that contained carcinogen were implanted into the right lower lobe bronchus. In dogs, for orthotopic carcinogenesis the carcinogens were repeatedly injected into the bronchial submucosa or topically applied to the bronchus; sustained release implants were implanted into the pulmonary parenchyma. Heterotopic focal canine bronchial carcinogenesis was accomplished by exposing s.c. bronchial autografts (8–12/dog) to methylcholanthrene.

In both species a predictable, reproducible, preneoplastic continuum that leads to bronchial squamous cell carcinoma that metastasizes has been characterized; serial measurements of total cellular DNA showed that ploidy increased in proportion to the stage of preneoplasia. In both species there were adenocarcinomas, including bronchial (bronchoalveolar) carcinomas and other varieties of non-small cell cancers. Different susceptibility to carcinogenesis has been demonstrated among different inbred strains of hamsters; 58% of cancers were adenocarcinomas in one strain. From these models, specimens that are not readily available from humans can be obtained for the study of cellular events during lung carcinogenesis. In parallel with studies in humans, these animal models can be used to evaluate methods of possible chemoprevention and early detection.

Introduction
In clinical practice, efforts to detect early lung cancers in high-risk patients with the use of screening sputum cytology failed to change therapeutic outcome results significantly (1). By current methods, one can predict neither which individuals will get lung cancer nor where such cancers might originate. Bronchogenic carcinomas are generally detected when a mass is seen radiographically or by bronchoscopy. When localized cancers are found, prompt excision is required, thus leaving little opportunity for experimental combined modality therapy of early bronchogenic carcinoma. In humans, there is currently no practical way to study or to attempt to arrest the sequential process of carcinogenesis.

Endobronchial carcinogenesis at focal sites, using local exposure to polycyclic aromatic hydrocarbons such as those found in cigarette smoke, has been the theme of our work since its initiation (2). Because of localization of the carcinogenic stimulus, we are able to induce bronchogenic cancers in hamsters and in dogs at predetermined sites, thereby simplifying the study of early carcinogenesis. We have been able to characterize the entire segmental progression of squamous cell cancers in dogs and hamsters. The time course of the process is now quite predictable in both species; the preneoplastic continuum of these lesions in hamsters and dogs is like that in humans. We have also produced adenocarcinomas and other NSCLCs (3), including bronchiolar carcinomas. The bronchial cancers we have induced in hamsters and dogs have culminated in distant metastasis, both in the autochthonous hosts and in tumor transplant models. Thus, the full spectrum of non-small cell bronchogenic carcinoma, from its preneoplastic beginnings to its systemic manifestations, is now available in animal models for sequential studies at the cellular level.

Materials and Methods
Carcinogen Administration Techniques. Intermittent exposure to carcinogens was accomplished in dogs by repeated submucosal injections via bronchoscopy (Fig. 1). Controls included the submucosal injection of saline without carcinogen, to ascertain the effects of recurrent focal trauma. Because repeated submucosal injection of carcinogen sometimes induced mucosal friability, additional intermittent carcinogen administration was accomplished by topical application. BP was the initial carcinogen employed; thereafter, N-methyl-nitrosourea was employed, either alone or in combination with BP. The efficacy and safety of various carcinogen regimens varied; the most recent experience showed that MCA provided the best balance of efficacy and safety. Details pertaining to intermittent carcinogen dose and administration techniques in dogs have been previously reported (3–9).

Continuous carcinogen administration has been accomplished in hamsters and dogs with the use of SRI. The vehicle for carcinogens has been room temperature-solidifying silastic polymer (Dow-Corning). Carcinogen, usually at a concentration of 10%, was mixed with the liquid silastic polymer; addition of the catalyst, stannous octoate, caused the mixture to harden in the desired form. Carcinogen was found to be released from SRI according to the first-order exponential functions. In hamsters we have used endobronchial SRI as solid cylinders; in dogs we have implanted endobronchial SRI as discs or as hollow cylinders. For intraparenchymal implantation, discoid SRI have been employed. In s.c. bronchial autografts, we injected carcinogen-containing liquid mixture before solidification was completed. The effects of control SRI (without carcinogen) have been evaluated in every location and in every form; such SRI have been found to be inert with respect to carcinogenesis. Details pertaining to the production and assessment of SRI have been previously reported (10–14).

Preparation in Hamsters. Cylindrical SRI, measuring approximately 3.5 mm in length and 1.1 mm in diameter, were inserted via tracheostomy into the right lower lobe bronchus, where they were retained by a fine wire hook included in the SRI (Fig. 2) (11). The desired endobronchial location was reliably achieved; dislocation of the SRI occurred rarely. It was possible to retrieve the SRI electrolytically means of an attached suture that was left under the skin adjacent to the trachea at time of initial placement. For retrieval, the hamster was anesthetized, repeat tracheostomy was done, and the SRI was removed by traction on the suture.

Preparations in Dogs. Orthotopic endobronchial carcinogenesis has been accomplished with repeated submucosal injections and topical applications of carcinogens via bronchoscopy. Endobronchial SRI, implanted as discs or cylinders, were frequently dislodged and found to be unreliable (6, 12).

Parenchymal carcinogenesis was accomplished during thoracotomies by placement of SRI into the lung parenchyma through short pleural tunnels.

2 Supported by Grants CA 26529 and CA 29373 from the National Cancer Institute of the NIH.
3 To whom requests for reprints should be addressed, at the UCD Medical Center, Professional Building, 4301 X Street, Sacramento, CA 95817.
4 SBA, s.c. bronchial autograft(s); SPC, sequential processes of carcinogenesis.

2687s
BRONCHOPULMONARY CARCINOGENESIS MODELS

LOBAR ORIFICE
INTERLOBAR SPUR
SHAFT OF NEEDLE
SUBMUCOSAL CARCINOGEN

Fig. 1. Submucosal endobronchial injection in dogs. A modified esophageal varix injection needle is used (A). The most commonly used site of injection of carcinogens has been the carina at the origin of the diaphragmatic lobe of the right lung. (B). (Reproduced from Ref. 9 with permission.)

incisions. Control SRI (without carcinogen) induced neither preneoplastic changes nor cancers.

Bistomal tracheal grafts were created by moving a 6-8-ring segment of trachea to a lateral s.c. location in the neck. Tracheal continuity was reestablished with end-to-end anastomosis; the isolated segment of tracheal mucosa was thereby available for biopsy and application of carcinogens. It was possible to replace the graft in its previous normal location to test reversibility of induced mucosal changes (7, 15).

SBA were created by implanting segments of autogenous bronchi, harvested after pneumonectomy, into the dorsal s.c. space of the same dog (Fig. 3). Normal ciliated bronchial epithelium was present by 3 weeks after implantation and was regularly maintained prior to addition of carcinogen. Carcinogenesis was initiated by placement of SRI into the SBA lumen during minor operations. The bronchial epithelium within the SBA could be repeatedly harvested, at intervals ranging from 3 weeks to several months (13, 14).

Assessment of the Carcinogenic Effects. Table 1 summarizes the methods of bronchial epithelial assessment which have been employed to date in five model systems; these are all like the methods used in the study of human lung cancer. The canine tracheobronchial epithelium was available for serial assessment by cytology, biopsy, and measurement of total cellular DNA in specimens from tracheal grafts, from orthotopic bronchial carcinogenesis, and from heterotopic bronchial epithelium in SBA (16–20). Fig. 4 shows a sheet of abnormal epithelium as well as examples of consecutive stages of the sequential progression of carcinogenesis (14). Sequential specimens could not be obtained from individual hamsters or from the same parenchymal carcinogenic implant in a dog. However, serial sacrifice experiments have been devised to assess the development of lesions in hamsters (21). In dogs, several parenchymal carcinogenic implants were placed at various locations within the lungs; these could be excised one at a time for study at appropriate intervals. Orthotopic lesions could be followed in both hamsters and dogs by serial chest X-rays (Fig. 5).

Results

Hamsters. The carcinogens BP and MCA have varying effectiveness, depending upon the dosage of carcinogen and the duration of exposure of the bronchial epithelium thereto. This prompted us to propose the term “family of cancers” (21). Other polycyclic aromatic hydrocarbon carcinogens have also been studied (22), but the model which has been most frequently used employs SRI with 10% BP. This results in focal cancers in >90% of animals about 150 days after SRI placement, when random-bred Syrian Golden hamsters are used (21). Only non-small cell carcinomas have been produced.

Fig. 2. Carcinogen-containing SRI in hamsters. In A, note hook that holds SRI in place in right lower lobe bronchus. In B, note cancer that developed at SRI site. All epithelial abnormalities have been at SRI sites. (Reproduced from Ref. 11 with permission.)

We showed that random-bred and inbred syngeneic F,D Syrian Golden hamsters had the same susceptibility to carci-


Fig. 3. SBA in dogs. A, normal ciliated bronchial epithelium; B, carcinoma. (Reproduced from Ref. 7 with permission.)

2688s
BRONCHOPULMONARY CARCINOGENESIS MODELS

Fig. 3. SBA in dogs. A. After pneumonectomy 12 autogenous bronchial segments are placed. B, Four weeks after implantation and before exposure to carcinogen. Note intact cartilage, normal submucosal glands, and normal ciliated surface epithelium with goblet cells. H & E, x 100. (Reproduced from Refs. 14 and 15 with permission.)

Carcinogen-altered bronchial epithelium was highly resistant to Adriamycin-induced lipid peroxidation, as compared to normal epithelium (25). We observed a 22% incidence of metastasis in autochthonous hosts with primary cancers of ≥10.0 mm in diameter (28). We have propagated hamster cancers in syngeneic hosts, in athymic nude mice, and in nude beige mice that have markedly depressed natural killer cell activity; there have been metastases from transplanted cancers in all varieties of xenotransplantation recipients. There was significant correlation (P < 0.05) between expression of the metastatic phenotype in the autochthonous host and the propensity of transplanted tumor to metastasize in the xenotransplant recipient. Moreover, there is evidence to suggest that the metastatic phenotype may be acquired and expressed during serial passage in xenotransplant hosts.

Dogs. Bilateral multiple parenchymal implants of SRI were well tolerated. The first parenchymal cancer occurred within 8 months after the beginning of the experiment (4). More recent evaluation showed that, in 7 of 12 dogs that each received carcinogen-containing SRI in three lobes, there were eight in situ squamous carcinomas, six microinvasive bronchiolar carcinomas, and one microinvasive acinar adenocarcinoma. It is noteworthy that adenocarcinoma and bronchiolar carcinoma were induced by parenchymal implantation and that there was also preneoplastic change as a result of parenchymal SRI.

Orthotopic carcinogenesis with submucosal injections of total MCA doses ranging from 540 to 1170 mg resulted in invasive cancers in about 18 (range, 12–24) months. This method evolved after trials of nine regimens which were either less effective or less well tolerated than the use of MCA (5, 6). Associated observations included the occurrence of pulmonary osteoarthropathy (29) and the demonstration of metastases according to a pattern which resembled that seen in humans with lung cancer (4).

Heterotopic bronchial carcinogenesis in SBA is our current method of choice in dogs. SBA epithelium can be serially harvested in sufficient amounts for molecular biological study; subsequent regeneration of the epithelium resumes at the stage of preneoplasm at which harvest was undertaken, i.e., sampling of an SBA that contains preneoplastic changes does not cause it to revert to normal epithelium during regeneration (14). We have had experience with 263 SRI-containing SBA in 25 dogs; these yielded 161 cancers. The cell types included 147 squamous cell carcinomas (7 in situ carcinomas and 15 that included the spindle-cell variant), 7 adenocarcinomas (2 with major bronchiolar components and 2 with major adenoid cystic components), and 2 poorly differentiated carcinomas. Two dogs have developed distant metastases from cancers arising in SBA. Using this preparation, we showed the severely atypical squamous metaplasia is reversible and not an inevitable forerunner of invasive cancer (30). The expense of a cancer induced by this method was estimated as $300, compared to more than $1000 for an orthotopic cancer.

Frustration was encountered during efforts to evaluate the effects of immunosuppression and old age upon the sequential progression of carcinogenesis (31). Dogs of age 7 years or older did not tolerate the carcinogenic regimens well (5). Even younger dogs did not tolerate the combination of azathioprine, corticosteroids, and carcinogen administration well enough to make us wish to continue this experiment (32).

In bistomal tracheal grafts, topical application and SRI of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Methods used in sequential study of bronchial carcinogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model system</td>
<td>Chest X-ray</td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
</tr>
<tr>
<td>Orthotopic carcinogenesis</td>
<td>+</td>
</tr>
<tr>
<td>Heterotopic carcinogenesis</td>
<td>-</td>
</tr>
<tr>
<td>Hamsters</td>
<td></td>
</tr>
<tr>
<td>Orthotopic carcinogenesis</td>
<td>+ (++)</td>
</tr>
</tbody>
</table>

*QDNA, total cellular DNA measured by image analysis (19).
Fig. 4. Samples of SBA epithelium. Cytological specimens, Papanicolaou, x 500; histological specimens, H & E; bars, 20 μm. A and B, regular squamous metaplasia, x 160. C, sheet of squamous metaplasia with atypia, obtained by curettagement. Note that most cells demonstrate a similar stage of atypia. D and E, moderately atypical squamous metaplasia. F and G, squamous cell carcinoma, x 300. (Reproduced from Ref. 14 with permission.)
carcinogens resulted in preneoplastic changes and in cancers (7). However, the results were relatively unpredictable and, therefore, we did not utilize this method further.

Heterotopic bronchial cancers have been carried in xenotransplantation in athymic nude mice and in nude beige mice. Current evidence indicates that the metastatic phenotype is maintained during growth in nude mice after transplantation from the autochthonous host. Metastases occurred in nude mouse recipients with equal frequency whether the tumors originated orthotopically or heterotopically (33).

Discussion

This summation of more than 1 decade of work with animal models of lung cancer is part of a symposium that is intended to foster work with chemoprevention and early detection of lung cancer in humans. Therefore, one must question the relevance of our findings to such investigations. Table 3 lists the characteristics by which our models resemble lung cancer in humans. As supplements to continuing studies in humans, we believe our lung cancer model systems offer opportunities that are not available in patients. Uniquely in our models, carcinogenesis occurs at selected focal site(s) that can be sampled at will at any stage of oncogenesis. Thus, the same trial could be conducted in patients and in animal models, thereby broadening the base of biological material available for studies at a cellular level.

Because the hamster model is relatively inexpensive and produces a high cancer yield from a single manipulation, it is particularly suitable for preclinical studies of chemoprevention or nutritional prevention methods. Because virtually all diagnostic methods applicable to humans for early detection can be evaluated and used repeatedly in dogs, the canine models are particularly suitable for preclinical investigations into new methods of early detection of lung cancer.

Additionally, canine models have the unique advantage that the bronchial epithelium can be harvested serially in relatively large amounts, without the death of the host. Moreover, it is now clear that virtually all types of non-small cell bronchogenic cancers can be induced in dogs, thus presenting an opportunity to study the preneoplastic continuum of adenocarcinomas and other lung cancers for which the early phases of the sequential progression of carcinogenesis are now poorly understood. A unitarian stem cell theory for bronchogenic carcinomas has been proposed (34). Although the merits of this theory need to be eventually evaluated in humans, the canine model can more efficiently be utilized for initial investigations.

We are cognizant and appreciative of the importance of neuroendocrine or Kulchitsky cell small cell lung cancers in experimental and clinical thoracic oncology (35-37). To date,
we have not seriously attempted to create neuroendocrine cancers. When we undertake this line of investigation, our models will have the advantage that they all employ carcinogenesis at a focal site that is readily accessible for study.

Acknowledgments

We appreciate the superb assistance we have received from various colleagues in Departments of Pathology and from our residents and research fellows throughout the many years of work summarized above.

References


Bronchial and Pulmonary Carcinogenesis at Focal Sites in Dogs and Hamsters

John R. Benfield and William G. Hammond

Cancer Res 1992;52:2687s-2693s.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/9_Supplement/2687s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.