Differential Susceptibility to Bronchial Carcinogenesis in Syngeneic Hamsters


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ABSTRACT

Previous studies of chemical carcinogenesis in the lung of Syrian golden hamsters have utilized outbred (nonsyngeneic) animals. Using the endobronchial sustained release implant technique, which causes focally originating cancers in outbred hamsters, we studied the course of bronchial carcinogenesis in two varieties of syngeneic Syrian golden hamsters, the LSH and the F1,D strains (BIO 15.16 male × BIO 87.20 female). With either 10% benzo(a)pyrene or 10% methylcholanthrene sustained release implants the time course of epithelial transition from normal to neoplastic was the same for F1,D hamsters as previously described for outbred hamsters. Using 10% benzo(a)pyrene sustained release implants the incidence of cancers as a function of time was significantly lower (P < 0.001) in LSH hamsters as compared to outbred and F1,D animals. Of 19 tumors transplanted into syngeneic F1,D hamsters, 16 have been successfully propagated by serial transplantation.

We conclude that (a) F1,D hamsters are comparable to outbred animals in the response of their bronchial epithelium to endogenous benzo(a)pyrene and methylcholanthrene, (b) there are significant differences in susceptibility to bronchial chemical carcinogenesis among hamster strains, thereby giving opportunity to study potential genetic control mechanisms during bronchial carcinogenesis, and (c) F1,D hamsters are suitable for studies of lung cancer biology using tumor transplantation methods.

INTRODUCTION

Syrian golden hamsters have a very low incidence of spontaneous pulmonary neoplasms, and they are susceptible to the carcinogenic effects of polycyclic aromatic hydrocarbons and nitrosamines.

To our knowledge, all previous studies of chemical bronchial carcinogenesis in Syrian golden hamsters have utilized outbred hamsters of various genetic backgrounds. Therefore, the possibility that various strains of hamsters may have differing susceptibilities to such carcinogens has not been explored, and the effects of genetic influences upon chemical bronchial carcinogenesis have not been assessed in detail.

We have developed a lung cancer model in hamsters which permits serial assessment of the stages in the progressive development of epithelial neoplasia at a preselected site; our model is based on endobronchial placement of a sustained release implant of carcinogen (1, 2). In past reports, we have described results in which outbred hamsters were used. The unpredictable and varying degrees of histocompatibility among such hamsters makes them poorly suited for experiments which utilize serial transplantations of lung cancer allografts. The following report describes studies of bronchial carcinogenesis in syngeneic hamsters and methods for in vivo propagation of cancers. The goal was to compare the rates of carcinogenesis between two strains of hamsters expected to have different susceptibilities to carcinogen and to achieve long-term propagation of lung cancers.

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MATERIALS AND METHODS

Two kinds of syngeneic Syrian golden hamsters were used for studies of carcinogenesis which were initiated when the animals were 8–12 weeks old: the LSH strain (Charles River Lakeview Laboratories, Newfield, NJ) and the F1,D hybrid (Bioresearch Institute, Cambridge, MA). The latter strain is the F1 hybrid from BIO 15.16 males and BIO 87.20 females (3). All hamsters were housed individually and fed standard laboratory chow and water ad libitum in a facility which has an approved and current USPHS Animal Welfare Assurance statement on file.

Carcinogens were administered via endobronchial sustained release implants which contained carcinogen; these shall be referred to as SRIs.2 The SRI contained either 10% BP or 10% MCA. They were prepared and implanted as previously described in detail (1). Finely powdered carcinogen was thoroughly mixed into liquid silicone polymer at 10% by weight. To initiate solidification of the polymer, the catalyst 0.5% stannous octoate was added. Immediately thereafter, while the mixture was still viscous, it was drawn into a 1.5-mm inside diameter glass tubing with a vacuum pump. After solidification of the polymer–carcinogen mixture, the glass was broken off, and the resultant carcinogen-containing silicone rod was cut into 3.5- to 4.0-mm lengths. A 4-0 stainless steel wire was passed axially through the cylinder and bent over to form a retaining hook at one end. Impaled upon a length of fine steel piano wire with the hook point ing rostrad, the implant was inserted into the right bronchus intermedius through a tracheotomy performed aseptically under light pentobarbital general anesthesia. The inserting wire was withdrawn, and the tracheal and skin incisions were closed individually with fine sutures.

BP, the primary carcinogen which had been used in previous studies of outbred hamsters, was used in LSH and F1,D hamsters. In addition, MCA was utilized in F1,D hamsters. The amount of carcinogen in SRIs placed in F1,D hamsters was 0.634–0.799 mg of BP or 0.612–0.757 mg MCA. The SRIs placed in LSH hamsters contained 0.636–0.795 mg of BP. We have noted no consistent dose-response effect over these ranges of initial carcinogen dosage. At 10% carcinogen in the SRI, the half-time of carcinogen release from BP SRIs into the bronchial lumen is 40 days and from MCA SRIs is 30 days (1, 2). Since release of carcinogen from the endobronchial SRI is a function of the gradient between carcinogen concentration at the SRI surface and carcinogen concentration in the immediately surrounding milieu (the intrabronchial mucus), no interstrain difference in rate of carcinogen release from the SRIs would be expected.

There were three experimental groups: 1, LSH hamsters which received 10% BP (n = 14); 2, F1,D hamsters which received 10% BP (n = 47); and 3, F1,D hamsters which received 10% MCA (n = 30). Hamsters of both sexes were used. We have found no difference between males and females in bronchial epithelial response to BP or MCA.

Starting 9–10 weeks after implantation of SRIs, F1,D hamsters were sacrificed in groups of 3–5 animals at 3-week intervals for the first 6 months and at 7- to 9-week intervals thereafter. Since LSH hamsters were expected to show a more prolonged course of carcinogenesis, two were sacrificed individually at 1- to 2-month intervals beginning 4 months after BP SRI implantation; a group of these was sacrificed at 160 days, and four others were individually sacrificed at approximately 3-month intervals thereafter. The study was terminated with sacrifice of the remaining seven animals at 400 days after SRI placement. In five instances, hamsters which appeared ill were sacrificed prior to the scheduled sacrifice dates.

At autopsy, the thoracic contents were removed en bloc. Tumors and pulmonary metastases which were visually apparent or were unseen but
palpable in the intact right lower lobe were excised and measured. After samples of each tumor were fixed in 10% formalin for histological examination, the remaining tumor was divided into two portions: one part was stored in sealed sterile containers at —70°C for later biochemical analysis, and the other portion was used for transplantation. When no tumor was palpable in the intact lobe, the bronchial segment which contained the SRI was opened longitudinally through the membranous portion. The SRI was removed, and the entire adjacent segment of bronchus was imbedded in 3% agar for orientation and then fixed in 10% formalin for subsequent histological examination. Lungs, mediastinal contents, or abdominal viscera which appeared grossly abnormal were also sampled for subsequent histological examination.

Specimens for histological examination were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. If no invasive cancer was noted on the initial sections, additional sections (1–2/100 μm of block thickness) were examined until either (a) invasive cancer was found or (b) representative sections from the entire bronchus adjacent to the SRI site had been studied. When no cancer was found, the most severe abnormality observed was taken as the end point of carcinogenic effect. This method reduces sampling error to a negligible level, and assures that the histopathological diagnosis for each hamster is definitive. In the 35 specimens wherein no cancer was initially found, an average of 29 (9–35) levels/specimen were examined by the multiple section technique.

Excluded from analysis were five hamsters that died and were unsuitable for adequate histological examination.

Statistical methods used included the Fisher exact test (4) for comparing incidences of histological change during comparable time periods; logistic regression (5), log linear fit to three-way contingency tables (6), and the log rank test (7) for comparing the time courses of occurrences of stages in the progressive development of cancer.

Transplantation experiments were done using primary or metastatic tumors from F,D hosts. A portion of each tumor was placed in a Petri dish filled with Dulbecco’s phosphate buffered saline (without divalent cations) containing penicillin, 0.5 mg/ml, and streptomycin, 5 mg/ml. All necrotic or infarcted tissue was trimmed away and discarded; the remaining viable tumor fragments were minced finely with scissors. Portions of tumor mince were placed s.c. in recipient hamsters, initially via a trocar, and later into a dorsal s.c. pocket developed by blunt separation through a short dorsal incision. The incision was closed with skin clips. Recipients were of both sexes and were 10–16 weeks old; we have found no sex difference in susceptibility to syngeneic tumor transplantation.

For subsequent transplants, the tumor was excised from its s.c. position and then minced and transplanted by the same methods. The variable amounts of tumor available from each preparation were divided among up to four hamsters available as tumor recipients. Samples were taken for histological examination from each transplant generation immediately after tumor removal. Especially in early transplant generations (first through fourth), a small portion of the s.c. tumor mass was left in situ with an intact blood supply to maintain the early transplant generation for repeated subsequent harvests.

RESULTS

SRIs were placed in 91 hamsters. Ten animals were excluded from the study: five hamsters that died from asphyxia due to dislodgement of the SRI within 2 weeks after placement, and five that died from unknown causes after 116–183 days of SRI exposure but prior to the times scheduled for their sacrifice. The tissues of the latter five hamsters were unsuitable for satisfactory pathological examination. Five hamsters that became ill were sacrificed prior to the time scheduled for sacrifice; these were at 200, 270, 273, and 392 days after SRI placement. All the remaining hamsters were in apparent good condition at time of sacrifice. Available for analysis were 81 hamsters: 12 LSH hamsters in Group 1 (10% BP-SRI); 45 F,D hamsters in Group 2 (10% BP-SRI); and 24 F,D hamsters in Group 3 (10% MCA-SRI) (Table 1).

Carcinogenesis. The sequential stages of pathogenesis in all the groups of hamsters (Fig. 1) were consistent with the neoplastic progression of epidermoid lung cancer which has been described and illustrated in detail previously (8). These phases are (a) squamous metaplasia, initially of a regular type (Fig. 2), later becoming progressively more atypical (Fig. 3), (b) carcinoma in situ (Fig. 4), and (c) invasive epidermoid cancer (Fig. 5). The invasive carcinomas are distinguished as being (a) detectable only at microscopy or (b) visually apparent tumors, which were minimal (>3 mm diameter), medium (3–10 mm diameter), or extensive (>10 mm diameter).

Of the 50 cancers which developed, 39 were typical squamous cell carcinomas, distributed among the three groups of hamsters without significant difference in incidence. The other neoplasms included three adenocarcinomas, two adenosquamous carcinomas, one poorly differentiated carcinoma, one spindle cell carcinoma, and one sarcoma in Group 2 (BP-F,D), 1 adenocarcinoma in Group 3 (MCA-F,D), and 2 adenocarcinomas in Group 1 (BP-LSH).

The time course for the development of cancer in Group 2 (F,D-BP) was compared to that in Group 3 (F,D-MCA) by the log rank test and was found not to be different (P > 0.1). The number of days of carcinogen exposure after which cancer was found in at least 85% of animals killed at that time for Group 2 (F,D-10% BP) was 154 and for Group 3 (F,D-10% MCA) was 176. In our previous study (2), the number of days of carcinogen exposure after which cancer was found in at least 85% of animals killed at that time in outbred hamsters was 140 for 10% BP-SRI and 175 for 10% MCA-SRI. These differences between F,D and outbred hamsters were not significant.

The time course of the progressive development of cancer observed in Group 1 (LSH-BP) was compared to that observed in the combination of Groups 2 and 3 (F,D-BP, F,D-MCA) and to that observed from our previous report in outbred hamsters which received 10% BP-SRI (2). Fig. 6 shows the comparison utilizing inverted Kaplan-Meier curves. Analysis of results by the log rank test and by logistic regression showed no difference between the time course of cancer development in F,D hamsters as compared to outbred animals. However, when compared by the log rank test and by logistic regression, the rate of occurrence of the progressive stages in the develop-
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Fig. 2. Regular squamous metaplasia of respiratory epithelium. H & E, × 375. Bar, 30 μm.

Table 1 Susceptibility to bronchial carcinogenesis

<table>
<thead>
<tr>
<th>Hamster strain/ carcinogen</th>
<th>Initially receiving SRI</th>
<th>Lost from SRI displacement</th>
<th>Died; carcass unsuitable for autopsy</th>
<th>Unscheduled sacrifice; clinically ill</th>
<th>Apparently well; scheduled sacrifice</th>
<th>Developed cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>F,D/BP</td>
<td>47</td>
<td>2</td>
<td></td>
<td>3</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>F,D/MCA</td>
<td>30</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>LSH/BP</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 3. Severely atypical squamous metaplasia; the abnormalities extend almost to the underlying cartilage at the bottom of the section. H & E, × 375. Bar, 30 μm.

Fig. 4. Carcinoma in situ. Note abnormal mitotic figures centrally and marked increase in thickness of epithelium as compared to Figs. 2 and 3. H & E, × 375. Bar, 30 μm.

ment of cancer as a function of time was lower \((P < 0.0001)\) in Group 1 (LSH-BP) as compared to Groups 2 and 3 (F,D hamsters). The slower progression in LSH hamsters was also demonstrated by a significant lack of fit in the log linear model for three-way contingency tables for all models not including the term for interaction between progression and time.

Additionally, the incidence of atypia plus cancer in F,D hamsters (11 of 12) sacrificed from 115–144 days after SRI placement was significantly greater \((P = 0.0004)\) than that in LSH hamsters after the same duration of carcinogen exposure (0 of 5). Furthermore, while the incidence of cancer after 175 days of exposure to carcinogen was 100% in F,D hamsters (39 of 39), the incidence of cancer in LSH hamsters was only 57% (4 of 7); this difference is significant at \(P = 0.000003\).

Tumor Propagation. Two squamous cell carcinomas which arose in LSH hamsters were transplanted into syngeneic hosts but failed to grow during 8 months of observation. Of 19 cancers from F,D hamsters transplanted into syngeneic hosts, 16 were successfully propagated; 3 failed to grow during periods of observation from 6–11 months. Fourteen epidermoid cancers, one adenocarcinoma, and one spindle cell carcinoma have been carried in serial transplantation for 4–8 transplant generations. Although growth rates of transplants vary from one tumor line to another, within each line the growth rate was dependent upon the dose of tumor cells transplanted. Among the 16 tumor lines, the time required for transplants to grow to >1 cm
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Fig. 5. Invasive squamous cell carcinoma. H & E, × 150. Bar, 60 μm.

Fig. 6. Inverted Kaplan-Meier curves showing time course for the development of cancer in F₁,D hamsters as compared to outbred (OB) hamsters and LSH animals. Percentages plotted reflect the number of hamsters with cancer as a function of the number of hamsters at risk of cancer at each time indicated.

Diameter ranged from 4–23 weeks; the mode was around 10 weeks. The histological patterns have not altered significantly with serial transplantation.

DISCUSSION

Until recently, Syrian golden hamsters used in laboratories were outbred descendants of three litter mates captured in 1930 (9). Beginning in the 1950s, a number of syngeneic strains of Syrian golden hamsters were developed. Biological phenomena which differ among inbred strains include the following: (a) responses of the circadian system to light changes (10); (b) susceptibility to experimental allergic encephalomyelitis (11); (c) normal values for certain serum chemical determinations (12); (d) susceptibility to dental caries (13); (e) the occurrence of benign prostatic hypertrophy (14); (f) longevity and reproductive performance (15, 16). Inbred strains have also been shown to have differing susceptibility to carcinogenesis from (a) the s.c. administration of polycyclic aromatic hydrocarbons (17); (b) the administration of MCA by gavage (18); (c) the inhalation of cigarette smoke (19); (d) α-radiation from 210polonium instilled into the trachea (20); and (e) the feeding of β-naphthylamine (21). Also, BIO 15.16 hamsters bind more than twice as much BP to tracheal epithelial DNA in vitro than do BIO 87.20 hamsters (22).

Both the BIO 15.16 and 87.20 parental strains and the F₁ hybrid of these two strains are susceptible to polycyclic aromatic hydrocarbon carcinogenesis by either p.o. or s.c. routes (3, 17, 18). Although the LSH hamster has not been used previously in studies of chemical carcinogenesis, this strain is relatively resistant to the oncogenic action of human adenovirus type 12 (23). The LSH strain and the BIO 2.4 strain both originated from the same stock, a colony brought from the London School of Hygiene to Philadelphia by Billingham. The BIO 2.4 strains has been shown to be relatively resistant to gastric and mammary carcinogenesis from p.o. MCA administration (17), although as a consequence of α-radiation from intratracheal instillation of 210polonium, this strain developed lung tumors earlier than did two other inbred strains which were more susceptible to chemical carcinogenesis than was the BIO 2.4 strain (20).

We recognize the analytic problems resulting from the relatively small numbers of LSH hamsters utilized in this initial exploration of chemical bronchial carcinogenesis in different hamster strains. While smaller sample sizes make it more difficult to achieve results in which differences have statistical significance, when statistical significance is indeed achieved, the relative smallness of sample size does not detract from the validity of such results. The rates of occurrence of the various stages in the progressive development of respiratory epithelial neoplasia were compared between LSH and F₁,D hamsters by four different independent statistical analyses, and all four showed a very high degree of statistical significance for the observed differences. Furthermore, the method of histopathological study used eliminates sampling error in diagnosis. Therefore, we consider that our findings indicate the existence of differential susceptibility to BP-induced bronchial carcinogenesis between LSH and F₁,D hamster strains. Additional studies are clearly required for confirmation and clarification of fundamental issues regarding potential differential mechanisms of pathogenesis.

Although epidemiological studies have shown population level exposure/effect relationships to lung cancer incidence, human lung cancer occurs without strict or predictable correlation with the individual extent of exposure to carcinogenic substances. Studies have not disclosed a basis for these interindividual variations in susceptibility; we believe they are most likely of genetic origin. Such recent reports as those which describe polymorphism of hepatic N-acetyltransferase among inbred hamster strains (24) and the finding of specific alterations in methylation patterns among lung cancers (25), taken with our demonstration of differential susceptibility to bronchial carcinogenesis among inbred strains, provide opportunity for in depth studies pertaining to factors which govern susceptibility to epithelial lung cancer.

Despite the extensive effort which has gone into epithelial lung cancer tissue culture experiments, regular success has not generally been achieved. This fact justifies pursuit of tumor transplantation methods. The successful serial transplantation of lung cancers in syngeneic hosts provides an in vivo method

4 R. Billingham, personal communication.
for propagation of tumor cells in quantities sufficiently large to permit biochemical analyses which are currently impossible on small amounts of tumor. Propagation by transplants among syngeneic hosts also enables studies of lung cancer biology which are free from the constraints of allogeneic homograft reaction.

The studies we have done in F,D inbred hamsters, in LSH hamsters, and in our previous reports using outbred hamsters (1, 2) show that the SRI approach to induce locally originating lung cancers is effective in all varieties of Syrian golden hamster tested to date. There is now evidence of differing susceptibility to standard chemical carcinogenic stimuli among strains of hamsters. Therefore, a variety of experimental models is now available for future studies of genetic control mechanisms during bronchial carcinogenesis.

REFERENCES


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