Effect of Chemical Carcinogens on Virus-induced Rous Sarcoma

Claire G. Engle and Vincent Groupé

Institute of Microbiology, Rutgers University, New Brunswick, New Jersey 08903

SUMMARY

In general, the effects of certain chemical carcinogens, known to be oncogenic in chickens, on virus-induced Rous sarcoma were not dramatic. Where cocarcinogenic effects were observed, they were most pronounced when the chemical carcinogen was injected after infection with Rous sarcoma virus. Cocarcinogenic effects were manifested primarily by more rapid tumor growth rather than by secondary tumor formation. Unusual tumor morphology was not observed. Of the carcinogens tested, 7,12-dimethylbenz(a)anthracene was the most effective cocarcinogen. N-2-fluorenylacetamide was cocarcinogenic when injected after virus but was inhibitory when administered prior to infection. Other samples enhancing virus-induced Rous sarcoma were 9,10-dimethyl-1,2-benzanthracene, benzo(a)pyrene, and 1,2,5,6-dibenzanthracene. 20-Methylcholanthrene was weakly cocarcinogenic.

INTRODUCTION

Reports on tumor induction in fowl following application or injection of hydrocarbons are numerous (2, 3, 11, 13, 14, 32, 36, 44-49, 52). Correlation of these published data is hindered by the multitude of variables represented within the experimental conditions, including age of bird, diluent employed, route of inoculation, concentration and regimen of treatment with the carcinogen, and the chemical structure of the hydrocarbon itself. The nature of carcinogenesis, whether it be mediated directly by the carcinogen itself (51) or by the activation of possible latent viruses within the host (22), has long been the subject of controversy. Theoretical significance of the latter interaction has prompted numerous investigators over the past 45 years to study the combined effects of chemical carcinogens with a variety of viruses. The additive or synergistic action of various carcinogens with different viruses has been observed in a number of animal species. This enhanced response was manifested in several ways: (a) enhancement of the acute proliferative and inflammatory effects of the virus (60); (b) enhancement of the neoplastic response of the host (1, 21, 23-26, 29, 34, 37, 40, 42, 50, 53-55, 58, 59, 61); and (c) localization of virus-induced Rous sarcoma at the site of carcinogen application (12).

On the other hand, chemical carcinogens and viruses do not always act in concert. The inhibitory effect of the carcinogenic polycyclic aromatic hydrocarbons, 7,12-dimethylbenz(a)anthracene and 3-methylcholanthrene, upon splenomegaly of Friend and Rauscher viral leukemias has been reported (27, 30). These hydrocarbons are known to depress cells of spleen and bone marrow, thereby interfering with the nonneoplastic components responsible for splenomegaly. Nevertheless, these investigators observed a prolonged latent period and survival time among the treated animals. Other evidence for direct inhibitory action of carcinogenic polycyclic aromatic hydrocarbons upon established neoplastic cells (33) and nontumor viruses (17) has been reported. In addition, the carcinogen, N-hydroxyurethan, has been shown to interfere with tumorigenesis both in vivo in the rabbit and in vitro by inhibiting the production of Shope fibroma virus (20).

A varied response to chemical carcinogens is not unexpected, for their modes of action undoubtedly differ. In addition, the number and nature of latent viruses present in the host at the time of application of the carcinogen is essentially unknown. Reports of cell-free transmission of chemically induced tumors of chickens (41) and rats (56) are in agreement with similar reports on cell-free transmission of radiation-induced leukemia in mice (31, 38). The data reported here summarize a preliminary survey designed to demonstrate additive synergistic effects of chemical carcinogens reported to be oncogenic for chickens (2, 3, 11, 13, 14, 32, 36, 44-49, 52) on host responses to Rous sarcoma virus (RSV) in chicks.

MATERIALS AND METHODS

Standard Rous Sarcoma Virus (RSV). A partially purified, standard preparation (43) of the Bryan strain of RSV was obtained from University Laboratories, Inc., Highland Park, New Jersey. A single, frozen stock, TV82, which was prepared from pooled tumor tissue by differential centrifugation, was employed throughout these experiments. TV82 was titrated in the remaining 3, (a) 9,10-dimethyl-1,2-benzanthracene (9, 10-DMBA), (b) 1,2,5,6-dibenzanthracene (1,2,5,6-DBA), and (c) 20-methylcholanthrene (20-MC) were acquired from K. &

1Aided by Grant #E-467 from the American Cancer Society.
2Present address: Microbiology Division, CIBA Pharmaceutical Company, Summit, New Jersey, 07901.
3Present address: Life Sciences, Inc., 2900-72nd Street North, St. Petersburg, Florida 33710.

Received October 7, 1968; accepted February 20, 1969.
K. Laboratories, Inc., Plainview, N. Y. Preparation of these compounds for injection was facilitated by pulverization in a mortar and pestle prior to suspension in 0.5% sodium carboxymethylcellulose (Grade 2 WXH). The sodium carboxymethylcellulose was kindly supplied by Hazleton Laboratories, Falls Church, Virginia.

**Test Procedure.** Unsexed white Leghorn chicks, 3 days of age, were obtained from Shamrock Farms, North Brunswick, New Jersey. A suspension of each carcinogen, prepared at the previously determined maximum tolerated dose (MTD), was inoculated in 0.2-ml quantities into the breast muscle of chicks 24 hours prior to, or 24 or 72 hours after, infection. The infection of 4-day-old chicks was accomplished by subcutaneous inoculation of 0.2-ml amounts of various concentrations of RSV into the wing web. Dilutions of RSV were prepared in physiologic saline containing 2% inactivated normal horse serum. Evaluation of the data was based on the following criteria: (a) total number and size of primary tumors at the termination of the experiment, (b) regression of primary tumors, (c) unusual tumor morphology, (d) production of secondary tumors at the site of carcinogen inoculation or elsewhere, and (e) Rankit analysis of the latent period for tumor production. Rankit (8, 35) represents probability units based upon the relationships of a normal curve and represents average deviations from the mean expressed in standard deviation units which are to be expected for a given number of observations within a common group. These values were ranked with respect to the time of onset of the primary wing tumor. Graphic methods have been shown by Bryan (4—9), permitting statistical analysis of the latent period for tumor production obtained in which wide fluctuations in susceptibility of the test-animal population of RSV are known to occur (5—7).

**RESULTS**

**Toxicity of Chemical Carcinogens for Chicks.** The data presented in Table 1 show the MTD of the 6 chemical carcinogens selected for study. In this experiment 4-day-old chicks, in groups of 10, were given a single injection of 0.2 ml of various concentrations of compound. The compound dilutions, in 2-fold increments ranging from 0.01% to 8%, were prepared in undiluted sesame oil and administered by 4 routes of inoculation: (a) intracerebral (i.c.), (b) intramuscular in the breast, (c) intraperitoneal, and (d) subcutaneous in the wing web. Each chicken was examined 6—7 times during the 16- to 18-day observation period. In general, death was the only criterion of toxicity. However, in some instances when a compound was injected intracerebrally or subcutaneously, local swelling was noted. This reaction, likewise, was regarded as a toxic response. The MTD of these hydrocarbons, as indicated in Table 1, was unchanged when the compound was suspended in 0.5% carboxymethylcellulose rather than in undiluted sesame oil.

Combined Action of RSV and Chemical Carcinogens. The common experimental chemical carcinogen, 20-methylcholanthrene, was selected for the study in the initial trials designed to demonstrate cocarcinogenic effects on host responses to RSV. The data obtained were inconclusive but are included here because of the importance of this compound. In 2 experiments, 2 common lots of 4-day-old chicks were each subdivided into groups of 15—20 chicks each. As indicated in Table 2, each chick was infected by subcutaneous inoculation into the wing web with 0.2-ml amounts of RSV diluted to 10⁻⁴ or 10⁻⁵. A single injection of a suspension of 20-methylcholanthrene, prepared at the previously determined MTD, was inoculated in 0.2-ml quantities into the breast muscle 24 hours prior to or 24 or 72 hours after infection as indicated. Control birds received a single injection of 0.5% carboxymethylcellulose alone 24 hours prior to infection. The onset and development of primary tumors were scored daily during the second week of infection and every 4 days thereafter for 3 additional weeks. An arbitrary scoring system based on the area of the wing web invaded by tumor tissue was used, e.g., wing web involvement was used: 1 = 10—25%; 2 = 25—50%; 3 = 50—75%; and 4 = 75—100% invasion. At the termination of the experiments, the chickens were autopsied and examined for secondary tumors. It will be seen from the data summarized in Table 2, that only in Experiment 2 was the incidence of tumor production increased in birds receiving 20-methylcholanthrene either before or after infection. However, 34 days after infection the size of the primary tumors in treated birds was less than that of the controls. With increased response of the test population of RSV, as illustrated in Experiment 1, the variation in incidence as well as the size of tumors decreased.

Because of the erratic results obtained with 20-methylcholanthrene, experiments were conducted in the same manner.
Table 2

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>RSV Dil.</th>
<th>Test substance</th>
<th>Conc. (%)</th>
<th>Injected (hr)</th>
<th>Tumor total</th>
<th>Latent period (days)</th>
<th>Av. tumor score* (31–34 days)</th>
<th>Secondary tumor</th>
<th>Regression ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10^-4</td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>18/20</td>
<td>7.5</td>
<td>3.9</td>
<td>0/14</td>
<td>1/18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>14/17</td>
<td>7.4</td>
<td>3.3</td>
<td>0/11</td>
<td>2/14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+24</td>
<td>16/20</td>
<td>7.2</td>
<td>2.9</td>
<td>0/14</td>
<td>4/16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+72</td>
<td>19/19</td>
<td>7.2</td>
<td>3.6</td>
<td>0/14</td>
<td>2/19</td>
</tr>
<tr>
<td>1</td>
<td>10^-5</td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>10/20</td>
<td>8.5</td>
<td>2.6</td>
<td>0/9</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>12/18</td>
<td>10.3</td>
<td>2.4</td>
<td>0/11</td>
<td>0/12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+24</td>
<td>16/20</td>
<td>9.6</td>
<td>2.9</td>
<td>0/15</td>
<td>0/16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+72</td>
<td>15/19</td>
<td>8.3</td>
<td>3.1</td>
<td>0/12</td>
<td>3/15</td>
</tr>
<tr>
<td>2</td>
<td>10^-5</td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>1/12</td>
<td>2.0</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMC</td>
<td>0.5</td>
<td>-24</td>
<td>6/15</td>
<td>2.8</td>
<td>0/5</td>
<td>0/0</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+24</td>
<td>7/15</td>
<td>2.3</td>
<td>1/5</td>
<td>1/7</td>
<td>1/7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-MC</td>
<td>4</td>
<td>+72</td>
<td>12/15</td>
<td>2.8</td>
<td>2/11</td>
<td>2/12</td>
<td></td>
</tr>
</tbody>
</table>


*Scoring system based on wing web invasion: 1 = 10–25%; 2 = 25–50%; 3 = 50–75%; 4 = 75–100%.

Table 3

<table>
<thead>
<tr>
<th>Test carcinogen</th>
<th>Conc. (%)</th>
<th>Codea</th>
<th>Injected (hr)</th>
<th>Tumor/total</th>
<th>Latent period Days</th>
<th>Potency</th>
<th>Av. tumor score* (35 days)</th>
<th>Secondary tumours</th>
<th>Regression ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAA</td>
<td>2%</td>
<td>10</td>
<td>-24</td>
<td>7/15</td>
<td>10.8</td>
<td>-2.1</td>
<td>2.8</td>
<td>0/4</td>
<td>1/17</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>+24</td>
<td>14/15</td>
<td>8.3</td>
<td>+2.4</td>
<td>3.1</td>
<td>0/4</td>
<td>3/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>+72</td>
<td>9/13</td>
<td>9.2</td>
<td>+0.6</td>
<td>3.0</td>
<td>0/3</td>
<td>3/9</td>
<td></td>
</tr>
<tr>
<td>9,10-DMBA</td>
<td>1%</td>
<td>13</td>
<td>-24</td>
<td>8/15</td>
<td>9.8</td>
<td>-0.4</td>
<td>3.9</td>
<td>0/3</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>+24</td>
<td>10/15</td>
<td>8.3</td>
<td>+2.6</td>
<td>3.7</td>
<td>1/1</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>+72</td>
<td>13/15</td>
<td>8.7</td>
<td>+1.6</td>
<td>4.0</td>
<td>0/6</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>1,2,5,6-DBA</td>
<td>1%</td>
<td>16</td>
<td>-24</td>
<td>9/14</td>
<td>9.1</td>
<td>+0.8</td>
<td>3.6</td>
<td>1/4</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>+24</td>
<td>11/14</td>
<td>10.0</td>
<td>-0.9</td>
<td>3.6</td>
<td>0/6</td>
<td>1/11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>+72</td>
<td>8/14</td>
<td>8.5</td>
<td>+2.0</td>
<td>3.0</td>
<td>0/5</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td>7,12-DMBA</td>
<td>1%</td>
<td>19</td>
<td>-24</td>
<td>5/13</td>
<td>9.5</td>
<td>0.0</td>
<td>4.0</td>
<td>0/2</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>+24</td>
<td>10/14</td>
<td>8.5</td>
<td>+2.0</td>
<td>4.0</td>
<td>0/3</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>+72</td>
<td>10/15</td>
<td>8.1</td>
<td>+3.0</td>
<td>4.0</td>
<td>0/3</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>4%</td>
<td>22</td>
<td>-24</td>
<td>9/15</td>
<td>9.8</td>
<td>-0.4</td>
<td>3.6</td>
<td>0/4</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>+24</td>
<td>9/15</td>
<td>8.3</td>
<td>+2.4</td>
<td>3.2</td>
<td>0/4</td>
<td>1/9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>+72</td>
<td>12/14</td>
<td>8.5</td>
<td>+2.0</td>
<td>3.3</td>
<td>0/3</td>
<td>1/12</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>0.5%</td>
<td>-24</td>
<td>5/12</td>
<td>9.5</td>
<td>0.0</td>
<td>2.7</td>
<td>0/2</td>
<td>1/5</td>
<td></td>
</tr>
</tbody>
</table>

Effect of selected chemical carcinogens on host response to RSV. FAA, N-2-fluorenylacetamide; 9,10-DMBA, 9,10-dimethyl-1,2-benzanthracene; 1,2,5,6-DBA, 1,2,5,6-dibenzanthracene; 7,12-DMBA, 7,12-dimethylbenz(a)anthracene; BP, benzo(a)pyrene; CMC, carboxymethylcellulose.

*aCode for graphic illustration (see Chart 1).
*bSee Table 2 for scoring system.

Using the following additional chemical carcinogens: (a) 7,12-DMBA, (b) 9,10-DMBA, (c) 1,2,5,6-DBA, (d) FAA, and (e) BP. The data obtained are summarized in Table 3. In this experiment a common lot of 300 chicks was subdivided into groups of approximately 15 chicks each. Chicks were infected with 1.0 ED₅₀ of standard RSV as previously described (7, 43), and each chick was given a single intramuscular injection of carcinogen as indicated. It will be seen that the incidence of tumor production among birds tested with these carcinogens was greater than that of the controls with one exception, i.e., in birds treated with 7,12-DMBA (this difference between the 2 samples of DMBA is probably attributable to the impurities present in the samples which were prepared by different laboratories) prior to infection. In addition, the size of the primary tumors was increased by the administration of each of these carcinogens. Rankit analysis of these data is graphically illustrated in Chart 1. In general, treatment prior to infection slightly delayed the latent
Carcinogen was delayed until 72 hours after infection with an oncogenic virus (RSV). The many and varied biologic activities of chemical carcinogens in vivo and in vitro are well documented and are too numerous for discussion. Alterations in the gross or histologic morphology of the tumors were not observed. Secondary or metastatic tumors were not enhanced, nor were secondary tumors observed at the site of injection of any of the chemical carcinogens tested. Virus-induced Rous sarcoma appears to be well suited for the detection of cocarcinogenic effects, particularly when the candidate preparation is injected after infection with a low dose of RSV but before the production of the primary tumor.

**REFERENCES**

18. DeMaeyer, E., and DeMaeyer-Guignard. J. Effect of Different Car-
Viral and Chemical Cocarcinogenesis


Effect of Chemical Carcinogens on Virus-induced Rous Sarcoma

Claire G. Engle and Vincent Groupé


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/29/7/1345

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.