Experimental Cancer of the Lung in Rabbits Induced by
Chemical Carcinogens

Fumio Hirao, Tomoo Fujisawa, Eiro Tsubura, and Yuichi Yamamura

Third Department of Internal Medicine, Osaka University Medical School, Dojima-Hamadori 3-chome, Fukushima, Osaka, Japan

SUMMARY

Lung cancer was induced in rabbits by the instillation of chemical carcinogens into the lower bronchus, with the use of a bronchoscope. The rabbits were divided into 2 groups. Group 1 received a mixture of 3-methylcholanthrene (3-MCA) and 4-nitroquinoline 1-oxide in rabbit plasma, and Group 2 received 3-MCA alone in distilled water. Doses of 40 mg of 3-MCA and 0.4 mg of 4-nitroquinoline 1-oxide were given every 7 to 10 days.

The total doses of carcinogens required for 50% incidence of lung cancer were 1000 ± 100 mg of 3-MCA and 10 ± 1 mg of 4-nitroquinoline 1-oxide in Group 1 and 1330 ± 100 mg of 3-MCA in Group 2.

Altogether, lung cancer was induced in 80 of 173 rabbits that received more than 4 doses of carcinogens and survived for more than 30 days. In 55 cases, metastases and/or invasion of the chest wall and diaphragm were observed. Histologically, the cancers were of various types (e.g., squamous cell carcinomas, adenocarcinomas, undifferentiated cell carcinoma, pleomorphic carcinomas, and mixed types).

The main bronchus, in rabbits of another group, was swabbed every 1 to 2 days with cotton wool soaked in a 10% suspension of 3-MCA in Tween 60. Of the 65 rabbits that survived for more than 60 days, only 2 developed lung cancer with metastases or invasion of tissues adjacent to the lung.

INTRODUCTION

We reported previously (12) on atypical squamous metaplasia and adenomatous proliferation that we induced in the bronchial submucosa of rabbits by swabbing the bronchus at intervals with a suspension of 3-MCA2 in Tween 60. The changes observed were limited to the submucosa and did not extend to, or cause destruction of, the cartilage. However, a few rabbits showed atypical squamous metaplasia and adenomatous proliferation in the peripheral bronchi and walls of the alveoli. These observations suggested that it might be possible to induce cancer in the parenchyma of the lung.

Accordingly, we devised the new technique of instilling carcinogens suspended in distilled water or rabbit plasma directly into the lower bronchus of rabbits. This resulted in a high incidence of typical lung cancer.

This report describes the technique used for induction of lung cancer and histological findings on the cancers that were induced.

MATERIALS AND METHODS

Young rabbits approximately 50 days old, weighing 1 kg, and adult rabbits approximately 100 days old, weighing 3 kg, both male and female, were used. The animals were given food (ORC 5, rabbit-guinea pig diet, from Oriental Yeast Industries, Ltd., Osaka, Japan) and water ad libitum throughout the experiment. The carcinogens used, 3-MCA and 4-NQO, were given in either normal rabbit plasma, distilled water, or Tween 60. 3-MCA, 4-NQO, and Tween 60 were obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Animals were anesthetized with ether (Sanraku Ocean Co., Ltd., Tokyo, Japan). A special bronchoscope (inner diameter, 4 mm; length, 20 cm) was used for instillation of the carcinogens.

Two experiments were performed. In Experiment 1, carcinogens were instilled into the bronchus, and in Experiment 2 carcinogen was swabbed on the inside of the bronchus. In Experiment 1, animals were divided into 2 groups. Group 1 contained 52 young rabbits and 63 adult rabbits, and Group 2 contained 18 young rabbits and 40 adult rabbits. Only animals that survived for more than 30 days were examined. Carcinogens were introduced into the right lung of all rabbits in Group 1 and into the right lung of 45 rabbits and the left lung of 13 rabbits in Group 2.

Rabbits in Group 1 received intrabronchial instillations of a mixture of 40 mg of 3-MCA and 0.4 mg of 4-NQO suspended in 0.4 ml rabbit plasma together with s.c. injections of a mixture of 0.1 mg of 4-NQO and 4 mg of cholesterol (Daiichi-Kagaku Co., Ltd., Tokyo, Japan) in olive oil (Wako Pure Chemical Industries, Ltd.). Group 2 received doses of 40 mg of 3-MCA alone, suspended in 0.4 ml of distilled water. The 1st 5 doses of carcinogens given to young rabbits were one half the amount given to adult rabbits. To instill the carcinogens, we anesthetized the animals and inserted a polyvinyl tube (diameter, 1.5 mm; length, 23 cm) into the lower bronchus through a bronchoscope. Instillations were repeated every 7 to 10 days.

In Experiment 2, the superficial mucosa of the right main bronchus of adult rabbits was swabbed with pieces of cotton soaked in a suspension of 10% 3-MCA in Tween 60, which was...
In Group 1, 40 mg of 3-MCA plus 0.1 mg of 4-NQO were instilled, and in Group 2, 40 mg of 3-MCA alone were instilled through a polyethylene tube into rabbits under ether anesthesia every 7 to 10 days. Group 1 also received 30 mg of 1,10-phenanthroline in olive oil. The doses of carcinogens required for 50% incidence of lung cancer were estimated as 100 ± 10 mg of 3-MCA and 0.2 ± 0.1 mg of 4-NQO in Group 1 and 130 ± 10 mg of 3-MCA in Group 2. In both groups, 50-day-old rabbits received one-half the dose given to adult rabbits in the 1st instillations.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Approximate initial age (days)</th>
<th>Chemical carcinogen instilled</th>
<th>Vehicle carcinogen + 4-NQO</th>
<th>Rabbit plasma</th>
<th>Rabbit plasma + 4-NQO</th>
<th>Distilled water</th>
<th>Distilled water + 4-NQO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3-MCA + 4-NQO</td>
<td>3-MCA + 4-NQO</td>
<td>3-MCA</td>
<td>3-MCA</td>
<td>4-NQO</td>
<td>4-NQO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>50</td>
<td>40</td>
<td>50</td>
<td>40</td>
<td>50</td>
</tr>
</tbody>
</table>

Hydrochloride (Pfizer Taito Co., Ltd., Tokyo, Japan), 50 mg/kg body weight, was given i.m. at the same time as were the carcinogens. Administration of carcinogens was continued unless the rabbits became severely ill or died.

Complete necropsies and histological examinations were done on rabbits that died or became severely ill during the experiment. All organs were examined macroscopically and were then fixed in 10% formalin. Sections of these tissues were stained with hematoxylin and eosin, as a routine method, and with periodic acid-Schiff, van Gieson stains, and silver impregnation, when necessary.

RESULTS

Experiment 1

Instillation of Carcinogens into the Lower Bronchus. Rabbits that received more than 4 instillations of chemical carcinogens and that survived for more than 30 days were examined histologically. Table 2 shows that administration of carcinogens reduced the survival rate. As seen from Table 1, 80 (approximately 46%) of 173 rabbits (90 females and 83 males) developed lung cancer. These included 42 cases of squamous cell carcinoma (approximately 50%), 22 cases of adenocarcinoma (approximately 28%), 6 cases of squamous cell carcinoma mixed with adenocarcinoma, and 8 cases of pleomorphic carcinoma. There was also 1 case each of undifferentiated cell carcinoma and sarcoma.

Various benign pathological changes were also observed (e.g., proliferation of atypical squamous epithelium of peripheral bronchioli, adenomatous hyperplasia, or adenoma), but only major pathological changes are recorded in Table 1. In this paper, adenomatous hyperplasia is defined as extensive hyperplasia extending from the terminal bronchi to alveoli, with an intact alveolar wall. Adenoma is defined as histological changes with formation of a glandular lumen lined by cylindrical or cubic cells, but without infiltrative changes or destructive growth.

The lung cancers showed various histological pictures. In some, squamous cells carcinomas cornification, necrosis, or somewhat poor differentiation was observed (Figs. 7 and 8). Most adenocarcinomas were composed of long cylindrical cells with eosinophilic cytoplasm and a basally located nucleus, but a few were composed of low columnar cells (Figs. 9 and 10). Scirrhous changes were often observed in both squamous cell carcinomas and adenocarcinomas. Many bizarre-shaped or giant cells were found in pleomorphic carcinomas (Fig. 11).

Most of the lung cancers were induced in the lower part of the lung on the side where the carcinogen was instilled, and they varied from 15 to 40 mm in diameter. In some rabbits, the lung was almost entirely replaced by tumor masses (Fig. 4). The center of the tumor masses was intensively necrotic (Figs. 2 and 3). In most rabbits the tumor adhered tightly to either the underlying diaphragm and/or the chest wall. Sometimes the diaphragm just below the tumor was so fibrotic that it was almost impossible to distinguish or separate it from the tumor mass.

Fixed onto the tip of a stick with the use of a bronchoscope. This treatment was repeated every day or every other day.

To protect rabbits from infection, oxytetracycline hydrochloride (Pfizer Taito Co., Ltd., Tokyo, Japan), 50 mg/kg body weight, was given i.m. at the same time as were the carcinogens. Administration of carcinogens was continued unless the rabbits became severely ill or died.
In 1 rabbit, a sagittal section of the right lung revealed disseminated tumors of from 1 to 25 mm in diameter (Fig. 1), and in the left lung many miliary metastases were also observed.

Metastases and Invasion of Tissues Adjacent to the Lung. Table 1 shows that, in 55 cases (approximately 70% of the total), metastases to other organs and/or invasion of tissues adjacent to the lung were observed.

Metastases were observed in the following areas: the paratracheal lymph nodes in 22 rabbits (Fig. 13); the kidneys in 7 rabbits (Figs. 6, 14); the mediastinum and chest wall in 4 rabbits (Fig. 5); and the collateral lung and diaphragm in 3 rabbits. There was 1 case each of metastasis to the liver (Fig. 16), adrenals, retroperitoneum, and omentum.

The diaphragm was the most frequently affected of the tissues adjacent to the lung (40 cases, i.e., 50% of the total). Cancer cells infiltrating the fibrotic muscle layer of the diaphragm were scirrhoid. Invasion of the muscle layer of the chest wall (Fig. 12) was noted in 14 rabbits.

Effects of the Dose of Chemical Carcinogen and Experimental Period on the Incidence of Lung Tumors. The minimum carcinogenic doses required for the induction of lung cancer were 380 mg 3-MCA and 3.8 mg of 4-NQO in Group 1 and 400 mg of 3-MCA in Group 2. On the basis of the relationship between the dose and the incidence of cancer, the doses required for 50% incidence of lung cancer production were estimated as 1000 ± 100 mg of 3-MCA and 10 ± 1 mg of 4-NQO in Group 1 and 1330 ± 100 mg of 3-MCA in Group 2.

In the early period of the experiment (within 60 days), the major pathological change was adenomatous hyperplasia, followed by the appearance of adenoma. No lung cancer developed by Day 60 after the 1st instillation of carcinogens. Metastases or invasion into tissues adjacent to the lung were 1st observed after Day 120 and were frequently found after Day 300.

Experiment 2

Swabbing the Bronchial Mucosa with Chemical Carcinogen. The bronchial mucosa was swabbed at intervals with a suspension of 10% 3-MCA in Tween 60. Only 2 of the 65 rabbits that survived for over 60 days developed lung cancer. These 2 rabbits were killed on Day 995 after the start of the experiment. In 1 rabbit, the lung to which the carcinogen had been applied was replaced by a tumor mass with extensive necrosis, and metastases were found in the paratracheal lymph nodes, retroperitoneum, and adrenals (Fig. 15), with invasion of the chest wall and diaphragm. Histologically, the cancer was defined as pleomorphic. In the other rabbit, the tumor mass was in the middle to lower part of the lung and invaded both the diaphragm and pleura. It was identified as a poorly differentiated squamous cell carcinoma.

DISCUSSION

It is difficult to induce lung cancer experimentally in animals, but recently there have been reports of the induction of lung tumors in mice, rats, and hamsters (1-11, 14-29, 31-38).

In humans, main bronchial cancer is the most common type of lung cancer. In an attempt to induce cancer in this site in rabbits, we repeatedly swabbed the main bronchus with a suspension of 3-MCA in Tween 60 (12). Repeated application of the carcinogen was presumed to be crucial for its prolonged action on the main bronchus. However, after more than 100 applications, many rabbits still showed no carcinomatous changes and the bronchial mucosa tended to show desquamation. However, some rabbits showed growth of atypical squamous epithelium and adenomatous hyperplasia in the terminal bronchi or alveolar walls. This suggested that the terminal bronchi or alveolar walls might be more susceptible sites.

Accordingly, we tested the effect of instilling carcinogens onto the lung parenchyma, using a polyvinyl tube inserted into the lower bronchus of rabbits. In a previous paper (13), which we believe was the first report of induction of lung cancer in rabbits, we stated that 5 of 32 rabbits developed lung cancer with metastases or invasion of neighboring tissue when carcinogens were instilled into the lower bronchus.

To obtain a high incidence of induction of lung cancer, a technique was required by which the carcinogens would remain in the lung for a long time. For this purpose, Andervont (1), Kuschner et al. (15) and Laskin et al. (16) tested the effect of sticking thread impregnated with carcinogen on the lung or of inserting pellets of chemical carcinogens or radioactive substances into the bronchi of mice and rats. The importance of the local persistence of the carcinogen was confirmed by Stanton and Blackwell (31), who induced multiple areas of pulmonary infarction in rats while giving them 3-MCA. Shabad et al. (30) administered carcinogen in a suspension with casein and India ink, while...
Saffiotti et al. (28) used crystals of ferric oxide to retain carcinogen in the lung for a long time.

The duration of the local persistence of a carcinogen seems to depend on the vehicle in which it is administered. Previously (12), we detected the autofluorescence of 3-MCA in the alveolar walls and terminal bronchi for as long as 10 days after its instillation, when given in distilled water or plasma. However, no autofluorescence could be detected after 10 days when 3-MCA was given in Tween 60. The reason for this may be that, when 3-MCA was given in distilled water or plasma, the latter fluids were rapidly absorbed so that insoluble crystals of 3-MCA were deposited on the walls of the alveoli and peripheral bronchi, while when 3-MCA was given in Tween 60, both were absorbed together into the circulation.

Previously, it was thought to be very difficult to induce experimental lung cancer in rabbits. However, induction is now possible by instilling 3-MCA, in either distilled water or plasma, into a given site in the lower bronchi through a bronchoscope.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful advice of Professor Tom Miyaji and Dr. Kunio Uematsu of the Department of Pathology, Osaka University Medical School, Osaka, Japan, and of Dr. Yasuyuki Akamatsu of the Department of Pathology, Medical College of Georgia, Augusta, Georgia.

REFERENCES

31. Stanton, M. F., and Blackwell, R. Induction of Epidermoid...

1212 CANCER RESEARCH VOL. 32


Fig. 1. Gross appearance of the right lung in a sagittal section, with disseminated tumors, 1 to 25 mm in diameter. This was classified histologically as a pleomorphic carcinoma. Rabbit P-133, female, Experiment 1, Group 1; experimental period, 529 days.

Fig. 2. Gross appearance of a primary squamous cell carcinoma, with intensive necrosis of the lower part of the right lung in sagittal section. This tumor adhered tightly to the underlying diaphragm and liver. Rabbit Y-104, male, Experiment 1, Group 2; experimental period, 524 days.

Fig. 3. Gross appearance of a primary squamous cell carcinoma of the right lung in sagittal section. The central and lower parts of the lung are occupied by tumor, and it is impossible to differentiate the diaphragm from the lung. Necrotic lesions are observed in the center of the tumor. Rabbit N-3, female, Experiment 1, Group 2; experimental period, 720 days.

Fig. 4. Gross appearance of a primary squamous cell carcinoma of the right lung in sagittal section. The tumor occupies almost all of the lung. There is extensive invasion of the tumor into the diaphragm (arrows). Rabbit P-47, male, Experiment 1, Group 1; experimental period, 399 days.
Fig. 5. Disseminated metastatic tumors, 2 to 8 mm in diameter, in the chest wall on both sides. *Rabbit P-133.*

Fig. 6. Gross appearance of the kidneys in sagittal section, with metastatic tumors, 1 to 10 mm in diameter. *Rabbit P-133.*

Fig. 7. Squamous cell carcinoma with intensive necrosis of the lung. *Rabbit Y-104.* H & E, x 100.

Fig. 8. Squamous cell carcinoma of the lung. *Lower portion of photograph,* squamous cancer cells infiltrating the pleura. *Rabbit L-25,* female, Experiment 1, Group 2; experimental period, 438 days. H & E, x 100.
Fig. 9. Tubular adenocarcinoma of the lung. Rabbit Y-17, male, Experiment 1, Group 1; experimental period, 368 days. H & E × 100.

Fig. 10. Tubular adenocarcinoma of the lung. In some areas of this specimen, pleomorphic structures are observed. Rabbit Y-37, male, Experiment 1, Group 1; experimental period, 724 days. H & E, × 100.

Fig. 11. Pleomorphic carcinoma of the lung showing bizarre-shaped and giant tumor cells. The tumor cells are extremely variable in size and shape. Rabbit P-133. H & E, × 40.

Fig. 12. Infiltration of squamous cancer cells from the lung into the muscle layer of the chest wall. Rabbit Y-104. H & E, × 100.
Fig. 13. Metastatic squamous cell carcinoma in the peripheral sinuses of the paratracheal lymph nodes. *Rabbit Y-75*, male. Experiment 1, Group 1; experimental period, 532 days. H & E, × 100.

Fig. 14. Metastatic squamous cell carcinoma in the kidneys. *Rabbit N-15*, female, Experiment 1, Group 2; experimental period, 644 days. H & E, × 100.

Fig. 15. Metastatic pleomorphic carcinoma in the adrenals. *Rabbit NM-13*, male, Experiment 2; experimental period, 995 days. H & E, × 100.

Fig. 16. Metastatic squamous cell carcinoma in the liver. *Rabbit Y-4*, male, Experiment 1, Group 1; experimental period, 480 days. H & E, × 40.
Experimental Cancer of the Lung in Rabbits Induced by Chemical Carcinogens

Fumio Hirao, Tomoo Fujisawa, Eiro Tsubura, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/6/1209

- **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.