Comparison between Carcinogenicity of N-Nitrosodiethylamine and Benzo(a)pyrene in the Respiratory Organs of Syrian Golden Hamsters when Induced by Intratracheal Instillation

Noburu ISHINISHI, Akiyo YAMAMOTO, Takeo INAMASU, Akira HISANAGA, Miyuki HIRATA and Shiro OYAMA

A comparison between the carcinogenicity of N-Nitrosodiethylamine (NDEA) and of benzo(a)pyrene [B(a)P] was carried out using male Syrian golden hamsters which were administered a total dose of 7.5 mg by means of intratracheal instillation once a week for 15 weeks. At the same time, a control group of hamsters were treated with a phosphate buffer solution. During their total life span, tumor incidence rates of the respiratory organs in the NDEA and B(a)P groups were 100% and 69% respectively, while in the control group it was 8%. With these results, it can be concluded that the carcinogenic potency of NDEA in the respiratory organs of Syrian golden hamsters is much higher than that of B(a)P.

Many species of natural and synthetic N-nitrosamines have been well known to be carcinogenic to several organs via several routes of administration in animal experiments. Recently, cyclic or non-cyclic volatile N-nitrosamines have begun to be considered as carcinogenic air pollutants to respiratory organs. On the other hand, it has long been suspected that B(a)P is one of the most carcinogenic of all air pollutants. Cadle and Mulawa reported that 0.1 ppb of NDEA was measured in the Eisenhower Tunnel in Colorado, and according to "Smoking and Health", the amount of NDEA and B(a)P in cigarette smoke is 0-20 ng/cigarette and 10-50 ng/cigarette respectively. In animal experiments, there have been many carcinogenic studies by means of intratracheal instillation concerning both NDEA and B(a)P. In our previous study, we reported that the carcinogenic potency of NDEA in the respiratory organs of Syrian golden hamsters was much higher than that of B(a)P when induced by intratracheal instillation. Concerning the intratracheal instillation method, it has been regarded as most appropriate for the investigation of carcinogenicity in the lung in animal experiments. In this report, reproducibility of comparative study between carcinogenicity of NDEA and B(a)P was proved using male Syrian golden hamsters and using intermittent intratracheal instillation.

Materials and Methods

N-Nitrosodiethylamine, a special grade, and phosphate buffer solution were obtained from Wako-Junyaku Co., Japan and B(a)P, a practical grade, was obtained from Sigma Ltd., Switzerland. These chemicals were used for the instillation experiment.

In 8-week-old male Syrian hamsters, whose body weights were 104-125 g, intratracheal instillation was carried out as previously described elsewhere. The hamsters were given atropine sulfate subcutaneously and then anesthetized with a mixture of 5% ether and 95% oxygen in a dessicator for 5 min.
Survival periods after 15 time instillation (month)

Fig. 1. Accumulative death rate (%) in effective numbers of hamsters.
1. NDEA —△—  2. B(a)P —○—  3. Control —□—

To the anesthetized hamsters, 0.5 mg of either NDEA solved or B(a)P suspended in 0.1 ml phosphate buffer solution, and to the control group, buffer solution alone was instilled once a week for about 15 weeks into the lung using a microsyringe with a special metal needle. The solution or suspension was homogenized and sterilized for 10 min. with an ultrasonic wave generator (Kaijo Denki Ltd., Japan) using nitrogen gas.

The experimental animals were divided into 3 groups: NDEA group, B(a)P group and control group as shown in Table 1. They were fed a commercial diet (Oriental MF, Oriental Co., Japan) and drinking water ad libitum. All these hamsters were observed during their entire life span. Those which died were autopsied and main visceral organs and any tissues or organs containing a tumor were fixed in 10% formalin solution. For histopathological examination, sections were prepared by a conventional method and stained with hematoxylin and eosin.

Results

All hamsters in the NDEA, B(a)P or control groups died within 232, 654 and 811 days respectively after initial instillation, and the accumulated death rates (%) of each group at monthly intervals are shown in Fig. 1. The cause of death during the instillation period in each group was due to pneumonia, besides one animal in the NDEA group, in which squamous cell carcinoma was manifested. Survivors after 15 intratracheal instillations in the NDEA, B(a)P and control groups were 14, 16 and 13 respectively of the 20 hamsters in each group as shown in Table 1.

In the NDEA group, tumors were detected in one or more respiratory organs in 14 of the 15 survivors examined. Thirteen lung tumors were observed in 12 animals. Ten of them were squamous cell carcinoma (Fig. 2), one of them was co-existence of squamous cell carcinoma and adenocarcinoma and one of them was an adenoma. Thirteen papillomas and one polyp in the trachea were found in the 14 survivors, and in eleven papilloma-bearing hamsters, there were co-existing malignant lung tumors, and in one polyp-bearing hamster, co-existing lung adenoma.

In the B(a)P group, tumors were detected in the respiratory organs of 11 of the 16 survivors examined. Two cases of squamous cell carcinoma, 4 adenocarcinomas, one undifferentiated carcinoma and one adenoma of the lung were detected in the 16 survivors. Four papillomas and one polyp of the trachea were observed in the 16 survivors. In three of the tracheal tumor-bearing hamsters, malignant lung tumors co-existed and in one of them adenoma of the lung co-existed.

In the control group, a lung adenoma was manifested in one of the 15 survivors. Neoplasms
Table 1. Tumor incidence in hamsters of the NDEA, B(a)P and control group.

<table>
<thead>
<tr>
<th>Group (dose)</th>
<th>Sex survivors after 15 instillations</th>
<th>Observation period following last instillation (days)</th>
<th>No. of examined hamsters</th>
<th>Lung tumor</th>
<th>Tracheal tumor</th>
<th>No. of tumor-bearing hamsters</th>
<th>Tumor incidence rate</th>
<th>Mean survival for tumor-bearing (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDEA (7.5 mg)</td>
<td>m 14/20 98-232</td>
<td>14 12 1 0 14</td>
<td>14(1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;d&lt;/sup&gt;</td>
<td>188</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(a)P (7.5 mg)</td>
<td>m 16/20 98-654</td>
<td>16 7 3 0 5</td>
<td>11</td>
<td>69</td>
<td>479</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (1.5 ml)</td>
<td>m 13/20 98-811</td>
<td>12(1)&lt;sup&gt;a&lt;/sup&gt; 0 1 0 0</td>
<td>1(2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>811</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>: No. of cannibalized hamsters.
<sup>b</sup>: No. of hamsters bearing squamous cell carcinoma in the lung, which died during instillation period.
<sup>c</sup>: No. of hamsters bearing neuroblastoma in the adrenal gland, hepatocellular carcinoma in the liver.
<sup>d</sup>: Significantly different from the B(a)P group (Logrank test, p<0.001).
M: Malignant.
B: Benign.

Fig. 2. Squamous cell carcinoma in the right lower lobule in the NDEA group.
The hamster died on the 182th day after the initial instillation. H.E. stain, ×190.

Other than respiratory tumors were neuroblastoma in the adrenal gland and hepatocellular carcinoma in the liver in the control group as shown in Table 1.

Besides lung tumor formation, squamous cell metaplasia of alveolar cell was seen in one animal of 14 survivors in the NDEA group. Tracheal cell hyperplasia, bronchiolar cell hyperplasia or papillary metaplasia of bronchiolar cell was seen in 2, 3 or one animal of 16 survivals in the B(a)P...
group. Broncholar cell hyperplasia was seen in 6 animals of 15 survivors in the control group.

The rates of tumor incidence in the respiratory organs in the NDEA and B(a)P groups were 100% and 69%, and the mean survival periods of tumor-bearing hamsters were 188 and 479 days, respectively. The difference in the rate between NDEA and B(a)P groups was significant using Logrank test (P<0.001).

Discussion

A series of experimental results have revealed that both NDEA and B(a)P are extremely powerful carcinogens to experimental animals. The interaction between intratracheally instilled B(a)P and subcutaneously injected NDEA in the hamster was investigated with positive results by Montesano et al. Furthermore, in our own previous study, we reported the carcinogenicity of NDEA in the respiratory organs of female hamsters to be of a higher rate than that of B(a)P.

In our present study, in order to prove the reproducibility of our previous study, we instilled NDEA and B(a)P at lower doses using male hamsters. The tumor incidence rate in the respiratory organs was 100% in the NDEA group and it was significantly higher than 69% in the B(a)P group. Furthermore, the mean survival period for tumor-bearing hamsters in the NDEA group was distinctly shorter than that in the B(a)P group as shown in Table I and in Fig. 1. Therefore, it has been made clear that the carcinogenic potency of NDEA given intratracheally to the respiratory organs of hamsters is significantly stronger than that of B(a)P, and that the trachea is more sensitive to tumor induction than the lung in the case of both NDEA and B(a)P at a certain given dose. These results confirm the reproducibility of a comparative study between the carcinogenicity of NDEA and B(a)P, where only a half dose was administered, the total dose of each drug being 7.5 mg. A surprising point to note, is that in this study, where the dosage was halved, and male rather than female hamsters were used, the tumor incidence rate in the B(a)P group was increased one and a half fold from 46% to 69%.

It has been recently recognized that airborne particulates contain considerable amounts of volatile N-nitrosamines and that polycyclic aromatic hydrocarbons (PAHs) in tarry materials, B(a)P in particular, are most responsible for tumor production in the respiratory organs. However, the contribution of NDEA to lung cancer has hardly begun to be evaluated either etiologically or epidemiologically at the present time. But following this study, we feel that NDEA as found in tarry materials obtained from airborne particulates has a carcinogenicity in the respiratory organs that should not be ignored.

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Key words: carcinogenicity, lung cancer, N-nitrosodiethy lamine, benzo(a)pyrene, animal experiment, intratracheal instillation