INHIBITORY EFFECT OF ALUMINIUM ON THE DEVELOPMENT OF EXPERIMENTAL LUNG TUMOR IN MICE INDUCED BY 4-NITROQUINOLINE 1-OXIDE

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Synopsis

The present series of experiment was undertaken to examine the effect of aluminium compounds on the development of experimental lung tumor induced by 4-nitroquinoline 1-oxide in mice. Female dd strain mice were injected subcutaneously once a week with 0.25 mg of 4-nitroquinoline 1-oxide suspended in 0.1 ml of 10% lecithin, for 5 weeks. Group 1: This group received no further treatment. Of 28 animals in this group, multiple lung adenoma developed in 100%, while three animals developed adenocarcinoma.

Groups 2 and 3: One week before the administration of 4-nitroquinoline 1-oxide, the animals were made to inhale 0.2% AlCl₃ solution (Gr. 2) or Al₂O₃ (Gr. 3) daily, and then further inhalation was continued twice a week for 7 months after 4-nitroquinoline 1-oxide treatment. In Groups 2 and 3, about 60% and 70% of the animals developed a small lung adenoma without development of adenocarcinoma. In the other experiment in which the animals were treated subcutaneously with AlCl₃ solution instead of inhalation in the same pattern, similar tendency was observed.

These experiments indicate that the presence of aluminium reduced the incidence of lung adenomas and suggest that aluminium inhibits the induction of adenomatous change in the lung of mice by 4-nitroquinoline 1-oxide.

INTRODUCTION

It was reported by Tipton et al., that the concentration of aluminium in human lungs increased markedly with age, whereas in other tissues, it remained at about the same level for all ages.

The abnormal accumulation of aluminium in human lungs was considered most likely to be due to atmospheric factors, such as air pollution. Biological effect of its administration seems not to have been studied much and not much is known of its mechanism of action in this respect.

The present investigations were started to explore the biological action of aluminium controlling the development of lung tumor by carcinogens. Our previous report showed that subcutaneous injection of 4-nitroquinoline 1-oxide with inhalation of aluminium produced typical lung adenoma in mice in a lower rate than in the group given 4-nitroquinoline 1-oxide alone, while a group given inhalation of aluminium showed the production of squamous lesion in a few cases.

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With reference to previous data, a series of experiments were then carried out by various routes in order to clarify the suppressive action of aluminium on the formation of lung adenoma in mice, as part of the analysis of mechanisms controlling atypical epithelial growth culminating in the formation of an adenoma as the reaction of bronchopulmonary epithelium against the carcinogenic substance.

In order to fully obtain the effect of aluminium at the time of subcutaneous administration of 4-nitroquinoline 1-oxide, aluminium was given every day for 1 week as a pretreatment, and then twice a week thereafter for an experimental period of 7 months.

**Materials and Method**

**Animals** Female dd strain mice of around 20 g in body weight were used. The animals were maintained on a basal mouse diet, with water ad libitum.

**Reagents**

0.2% Aluminium (AlCl₃) Solution: A solution of 1.7894 g AlCl₃•6H₂O (Koso Chem. Co., Ltd.) dissolved in 100 ml of water.

Alumina Powder (Al₂O₃): Activated Al₂O₃ (for chromatographic adsorption analysis, Merck) was reduced to a fine powder.

Aluminium Injection Solution: A mixture of 20 ml of 0.2% AlCl₃ solution and 2.0 g of animal lecithin (Merck) was heated in a boiling water bath for 15 min, cooled, and used immediately.

4-Nitroquinoline 1-Oxide Injection: A homogeneous mixture of 50 mg of 4-nitroquinoline 1-oxide, 20 ml of water, and 2.0 g of lecithin was heated in a boiling water bath for 15 min, cooled, and used immediately.

4-Nitroquinoline 1-Oxide + AlCl₃ Solution: A homogeneous mixture of 30 ml of 0.2% AlCl₃ solution, 75 mg of 4-nitroquinoline 1-oxide, and 3.0 g of lecithin was heated in a boiling water bath for 15 min, cooled, and used immediately.

**Inhalation Procedure**

a) Inhalation of Aluminium Solution: Ten mice each were maintained in an inhalation chamber with a capacity of 14000 cm³ and 3 ml of 0.2% AlCl₃ solution was introduced by a nebulizer continuously for ca. 40 min.

b) Alumina Inhalation: Ten mice were placed in the same chamber and 300 mg of finely powdered Al₂O₃ was evaporated by a nebulizer compressor for about 50 min.

**Subcutaneous Injection** For 4-nitroquinoline 1-oxide, 4-nitroquinoline 1-oxide + AlCl₃, and AlCl₃ solution, a single dose of 0.1 ml (0.25 mg of 4-nitroquinoline 1-oxide) was injected at different sites on the back of mice.

**Determination of Aluminium in the Lung** Determination was carried out with reference to Sandell's colorimetry for aluminium. Mice were anesthetized with ether, physiological saline was injected directly into the heart to remove blood in the lung, desanguinated lung was exirpated, blotted on a filter paper, and weighed. The lung was placed in a Kjeldahl flask, 2 ml of H₂SO₄ and 5 ml of HNO₃ were added, and heated with occasional addition of HNO₃ until the liquid became colorless and clear. The decomposed solution was diluted with water to about 6N H₂SO₄; iron was removed by extraction with Cupferon and CHCl₃, and the amount of aluminium in the solution was determined by colorimetry at 525 mμ, using the aluminon reagent.
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**Experimental Groups** The aluminium administered groups were divided into inhalation group (Group A) and injection group (Group B).

- **Group A-1:** Initial 1st week was not treated. From the 2nd week of experiment, 0.25mg of 4-nitroquinoline 1-oxide was injected subcutaneously once a week for 5 times, to a total of 1.25mg of the chemical.
- **Group A-2:** 4-Nitroquinoline 1-oxide was administered in the same manner as in Group A-1, preceded by daily inhalation of aluminium solution for 1 week, followed by inhalation twice a week for 7 months during and after subcutaneous injection of 4-nitroquinoline 1-oxide.
- **Group A-3:** Same treatment as Group A-2 but with inhalation of Al₂O₃ in place of AlCl₃ solution.
- **Group A-4:** Inhalation of AlCl₃ solution alone, without injection of 4-nitroquinoline 1-oxide.
- **Group A-5:** Inhalation of Al₂O₃ alone, without injection of 4-nitroquinoline 1-oxide.
- **Group A-6:** Untreated control.

- **Group B-1:** Same as Group A-1, with subcutaneous injection of a total of 1.25mg of 4-nitroquinoline 1-oxide.
- **Group B-2:** Same as Group B-1 with 4-nitroquinoline 1-oxide + AlCl₃, making a total of 1.25mg of 4-nitroquinoline 1-oxide.
- **Group B-3:** Same as Group B-2, preceded by subcutaneous injection of AlCl₃ solution three times a week for 1 week, followed by twice a week with the same treatment during and after subcutaneous injection for 7 months.
- **Group B-4:** Subcutaneous injection of 0.1ml of AlCl₃ (0.2mg) solution, twice a week for 7 months.
- **Group B-5:** Untreated control.

**RESULTS**

Several animals were lost at the initial stage of the experiments due to acute intoxication of 4-nitroquinoline 1-oxide or by asphyxiation by aluminium inhalation, pneumonia, etc. Lung tumors were classified histologically into the following three types¹):

- **Typical Multiple Adenoma:** The tumor cells were low cuboidal or ovoid, lying in a thin alveolar stroma and occasionally there existed the pattern of papillary type (Photo 2).
- **Malignant Adenoma:** Adenomatous tumors with marked atypical cell proliferation without destructive multiplication (Photo 3).
- **Adenocarcinoma:** The cells have extensive atypical proliferation and became irregular in size and shape showing papillary formation, and invaded the lung parenchyma (Photo 4). Their nuclei were more hyperchromatic than those of malignant adenoma.

Occurrence of lung tumor in the mice, as shown in Table I, was clearly lower in the group given aluminium inhalation than in the groups given 4-nitroquinoline 1-oxide alone, especially in the production of lung adenoma which occurred in 100% of the mice given the chemical alone, while the incidence was 64% in the animals.
given AlCl₃ at the same time and 75% in that given Al₂O₃ at the same time. In the group given 4-nitroquinoline 1-oxide alone, invasive and destructive adenocarcinomas were found in three cases, while none was observed in the animals given aluminium.

The number of nodules on lung surface due to lung tumor was less numerous and smaller in the group given aluminium than in that given the chemical alone. The average number of surface nodules was 20.3 in the group given 4-nitroquinoline 1-oxide alone (Photo 1), while the number was 10.5 in Group A–2 and 7.3 in Group A–3, clearly showing the suppressive effect of aluminium.

In experimental group B’s (Table II), lung adenoma was observed in 26 out of 28 cases (92.9%) of the group given 4-nitroquinoline 1-oxide alone, while the occurrence was 15 out of 31 cases (48.4%) in Group B–2 and 11 out of 22 cases (50%) in Group B–3, in which average number of nodules on the surface was 2.3 and 2.0 against 7.4 in the former group. Size of the nodules was also smaller in the groups given aluminium.

Determination of aluminium content in the lung of experimental animals showed that accumulation of aluminium in the lung was about 2~3 times higher in the inhalation group than in non-inhalation group, while such a difference was not observed in the experiment B in which aluminium was administered by subcutaneous injection (Table III).

<table>
<thead>
<tr>
<th>Group</th>
<th>Init. No. of mice</th>
<th>Effect. No. of mice</th>
<th>Incidence of lung tumor (%)</th>
<th>No. of nodules (medium)</th>
<th>Histological findings</th>
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<tr>
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<td>27</td>
<td>27 (100.0)</td>
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<td>16 (64.0)</td>
<td>10.5</td>
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<td>24</td>
<td>18 (75.0)</td>
<td>7.3</td>
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<td>40</td>
<td>25</td>
<td>1 (4.0)</td>
<td>0.04 (1/25)</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>22</td>
<td>0 (0)</td>
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Table I. Lung Findings in the Experimental Group A

<table>
<thead>
<tr>
<th>Group</th>
<th>Init. No. of mice</th>
<th>Effect. No. of mice</th>
<th>Incidence of lung tumor (%)</th>
<th>No. of nodules (medium)</th>
<th>Histological findings</th>
</tr>
</thead>
<tbody>
<tr>
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<td>26 (92.9)</td>
<td>7.4</td>
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<td>15 (48.4)</td>
<td>2.3</td>
<td>15</td>
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<tr>
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<td>11 (50.0)</td>
<td>2.0</td>
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<td>35</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
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<td>5</td>
<td>30</td>
<td>21</td>
<td>0 (0)</td>
<td>0</td>
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</tr>
</tbody>
</table>
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Table III. Average Amount of Aluminium in Lung

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group A-6)</td>
<td>6.5</td>
<td>6.0</td>
<td>6.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Al injection (Group B-4)</td>
<td>6.5</td>
<td>6.3</td>
<td>5.5</td>
<td></td>
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<tr>
<td>Al inhalation (Group A-4, 5)</td>
<td>12.0</td>
<td>14.0</td>
<td>16.1</td>
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DISCUSSION

The results of the experiments presented here indicate that the inhalation of aluminium in combination with subcutaneous injection of 4-nitroquinoline 1-oxide can interfere with the mechanisms of induction of lung adenoma and adenocarcinoma in mice, at least within the period of the present experiments.

In the experimental production of lung tumor in mice by the subcutaneous injection of 4-nitroquinoline 1-oxide alone, adenomatous tumor and its type of growth are the general form of the reaction of bronchopulmonary epithelium by the carcinogen,3-5) and nothing is known of the mechanisms that make production of squamous pattern difficult.

These experiments confirm the suppressive effect of aluminium on the induction of lung tumor by 4-nitroquinoline 1-oxide and indicate that this effect can be obtained even when aluminium is given subcutaneously.

As was found in the present series of experiments, aluminium seems to be a factor that interferes with an adenomatous change, and it should be noted as affecting the mechanisms of the carcinogen that produces tumorous change in the respiratory epithelium.

The fact that the inhalation of AlCl₃ solution or Al₂O₃ powder seems to suppress the carcinogenicity of 4-nitroquinoline 1-oxide should probably be considered as the action of aluminium itself rather than the acidity (pH) of AlCl₃ or the biological reaction of Al₂O₃ powder attributable to the physical characteristics of the powder as a foreign substance.

Further, suppressive action of lung carcinogenesis by 4-nitroquinoline 1-oxide even by subcutaneous injection of AlCl₃ may be assumed to be the specific biological action of aluminium on the metabolism of 4-nitroquinoline 1-oxide at the site of injection, i.e., abnormal metabolism, which lessens the effect of carcinogen on the lung, or to the interaction of aluminium with 4-nitroquinoline 1-oxide in the lung which works in the same way as in inhalation of aluminium.

It is expected that the extension of these studies will permit a closer investigation of the interplay between lung carcinogenesis and aluminium element in controlling cellular differentiation and the development of tumors in bronchopulmonary epithelium.

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REFERENCES


EXPLANATION OF PLATES XL~XLI

Photo 1. Multiple lung tumors of a mouse, induced by 4-nitroquinoline 1-oxide (Group A-1).
Photo 2. Photomicrogram of a typical multiple lung adenoma (Group A-1). H-E. × 100.
Photo 4. Subpleural extension of papillary adenocarcinoma induced by 4-nitroquinoline 1-oxide (Group A-1). H-E. × 100.

H-E=Hematoxylin-Eosin stain.