EFFECT OF ALUMINIUM CHLORIDE ON METABOLISM OF 4-NITROQUINOLINE 1-OXIDE*1

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Aluminium chloride was subcutaneously administered to mice and its effect on the activities of 4-nitroquinoline 1-oxide (4-NQO) reductase and 4-hydroxyaminoquinoline 1-oxide (4-HAQO) reductase, and the organ distribution of carcinogen(s) in mouse lung and liver were examined.

Subcutaneous administration of aluminium chloride in mice results in significant elevation of 4-NQO reductase and 4-HAQO reductase activities in their lung and liver, compared with those of the control.

Simultaneous subcutaneous administration of aluminium chloride with 4-nitroquinoline[5,6,7,8,9,10-14C] 1-oxide (14C-4-NQO) and examination of radioactivity distribution in the lung and liver showed that the radioactivity per tissue, 0.5 and 1 hr after the administration, decreased in the lung but inversely increased in the liver. Radioactivity in the lung and liver 2 hr after the administration was not different from that of the control.

Simultaneous subcutaneous administration of 14C-4-NQO and aluminium chloride resulted in decreased distribution of 4-NQO and 4-HAQO in the lung compared with that of the control, while the distribution of their metabolites, 4-aminoquinoline 1-oxide (4-AQO) and 4-hydroxyquinoline 1-oxide (4-OHQO), inversely increased. Distribution of 4-aminoquinoline (4-AQ) and 4-hydroxyquinoline (4-OHQ) in the lung was not different from that of the control.

These results suggest that the rapid metabolic changes of carcinogenic 4-NQO and 4-HAQO to noncarcinogenic substance(s) and decrease in the concentration of carcinogenic substances in the lung by the subcutaneous administration of aluminium chloride constitutes one of the factors for the mechanism of the suppression of carcinogenesis by aluminium chloride.

Subcutaneous administration of 4-nitroquinoline 1-oxide (4-NQO) to mice results in the production of lung cancer in a high incidence6–8) but we have previously found that simultaneous inhalation of aluminium oxide or subcutaneous injection of aluminium chloride suppresses the production of lung cancer by 50% or so.2,3) We have shown in our preceding paper11) that aluminium chloride suppresses the binding of carcinogenic substances, 4-NQO and 4-hydroxyaminoquinoline 1-oxide (4-HAQO), to nucleic acids in the lung especially, and suggested that this is one of the factors in the mechanism of carcinogenesis suppression by the subcutaneous administration of aluminium chloride.

In the present series of the work, variation in the activities of 4-NQO reductase and 4-HAQO reductase by the subcutaneous administration of aluminium chloride, and the mode of distribution of carcinogenic substances in the tissue were examined using

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*1 This constitutes Part II of a series entitled "Possible Mechanism of Suppressive Action of Aluminium Chloride on Lung Carcinogenesis in Mice induced by 4-Nitroquinoline 1-Oxide." Part I.11)

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the mouse lung, which is the target organ of 4-NQO carcinogenesis, and the liver, which is an important organ in drug metabolism.

**Materials and Methods**

**Animals** Male dd strain mice, 4\/~5 weeks of age and weighing 21\/~23 g, were used. The animals were fed on a solid food CE-2 (CLEA Japan, Inc., Tokyo) and given water freely. **Chemicals** 4-NQO was obtained from Wako Pure Chemical Ind., Ltd., Tokyo, and recrystallized from acetone. 4-HAQO, 4-AQO, 4-OHQO, 4-AQ, and 4-OHQ were synthesized by the method of Ochiai and others, and purified. 14C-4-NQO was the product of Daiichi Pure Chemicals Co., Tokyo, and had a specific activity of 6.22 mCi/mmol.

AlCl₃ solution was a homogeneous suspension of 125 or 500 mg of AlCl₃•6H₂O (Koso Chemical Co., Tokyo) in M/15 phosphate buffer (pH 7.0) diluted to 100 ml. 14C-4-NQO solution was prepared by the addition of 10 ml of water and 100 mg of 14C-4-NQO (1 μCi/mg) to 1.0 g of egg yolk lecithin (Merck product), warming the mixture in a water bath for 15 min with stirring, and cooled to room temperature. Both solutions were used immediately after preparation.

**Preparation of Lung and Liver Enzymes** Mice were divided into 3 groups of 6 animals each; experimental group Nos. 1 and 2 given 125 or 500 μg of AlCl₃, and the control group No. 3. In group 1, 1.25 mg/ml of AlCl₃ solution was injected subcutaneously on the back of mice in a dose of 0.1 ml/mouse, and in group 2, 5.0 mg/ml of AlCl₃ solution was injected subcutaneously on the back of mice in a dose of 0.1 ml/mouse. In the control group, M/15 phosphate buffer (pH 7.0) was injected subcutaneously in place of AlCl₃ solution.

Mice were killed by cervical dislocation 2 hr after the subcutaneous injection of AlCl₃ solution, ice-cold 1.15% KCl-0.01M phosphate buffer (pH 7.4) was injected directly into the heart to remove blood in the lung and liver, and the lung and liver were rapidly removed. The organs were washed with ice-cold 1.15% KCl-0.01M phosphate buffer (pH 7.4), and blood and moisture were wiped off. Each organ was minced finely, and homogenized with the same buffer to make a 25% (w/v) homogenate by a glass homogenizer. After centrifugation at 750 g for 20 min, the supernatant was recentrifuged at 10,000 g for 20 min at 5° and the supernatant (10-S) was collected.

**Measurement of Enzyme Activity**

4-NQO Reductase Activity: The supernatant (10-S) from the lung and liver was each diluted with M/15 phosphate buffer (pH 6.6) to prepare 12.5% and 5% solution, respectively. To 0.9 ml of each of these solutions, a solution of 1 μmol (50 μl) of NADP, 5 μmol (50 μl) of glucose-6-phosphate (G6P), and 20 μmol (100 μl) of nicotinamide dissolved in M/15 phosphate buffer (pH 7.0) was added, followed by 4 μmol (200 μl) of 4-NQO dissolved in methanol, and the mixture was incubated at 37° for 30 min.

At the end of incubation, 5.0 ml of benzene was added to the mixture, unchanged 4-NQO was extracted by shaking, and its amount was determined. Activity of 4-NQO reductase was measured from the residual amount of 4-NQO. Protein was determined by the method of Lowry et al., using bovine serum albumin as standard.

4-HAQO Reductase Activity: The 25% 10-S solution from the lung and liver was each diluted with 0.5M phosphate buffer (pH 8.5) to prepare 12.5%, 10-S solution. To 1.0 ml of each of these solutions, 1 μmol (50 μl) of NADP, 5 μmol (50 μl) of G6P, and 20 μmol (50 μl) of nicotinamide were added, followed by a solution of 500 μg (100 μl) of 4-HAQO hydrochloride dissolved in methanol, and the mixture was incubated at 37° for 60 min. To this incubated mixture, 0.1 ml of 4M HCl and 3.0 ml of CHCl₃ were added, the mixture was shaken vigorously, and centrifuged at 3000 rpm for 10 min. For thin-layer chromatography, 200 μl of this supernatant was applied in a band on a thin-layer plate (silica gel 60, 0.25 mm thickness, Merck) and the plate was developed with a solvent system of sec-BuOH:AcOEt:H₂O (2:1:1, v/v). The band corresponding to 4-AQO (Rf 0.35), a metabolite of 4-HAQO, was scraped off from the thin-layer plate and its amount was determined. Activity of 4-HAQO reductase was calculated from the amount of 4-AQO formed.

**Distribution of Radioactivity in the Lung and Liver after Simultaneous Administration of 14C-4-NQO and AlCl₃** Mice were divided into two groups of 24 animals each; one group was given AlCl₃ and 14C-4-NQO, and the other was given 14C-4-NQO alone as a control. For the experimental group, 5 mg/ml of AlCl₃ solution was injected subcutaneously on the left back in a dose of 0.1 ml/mouse, and 0.1 ml/mouse of 14C-4-NQO (10 mg/10 μCi/ml, as egg lecithin suspension) was injected subcutaneously on the right back at the same time.

Six mice were killed by cervical dislocation at each of 0.5, 1, 2, and 4 hr after the administration, ice-cold 1.15% KCl-0.01M phosphate buffer.
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(pH 7.4) was injected directly into the heart to remove blood in the lung and liver, and the lung and liver were rapidly removed. The organs were washed with ice-cold 1.15% KCl-0.01M phosphate buffer (pH 7.4), and blood and moisture were wiped off. Each organ was dried overnight at room temperature.

Radioactivity per tissue was measured with a sample oxidizer (Packard, Model 306) and a liquid scintillation spectrometer (Packard, Model 3320).

**Distribution of 14C-4-NQO and Its Metabolites in the Lung and Liver after Simultaneous Administration of 14C-4-NQO and AlCl₃**

Mice were divided into two groups of 6 animals each; one group was given AlCl₃ and 14C-4-NQO, and the other was given 14C-4-NQO alone as a control. For the experimental group, 5 mg/ml of AlCl₃ solution was injected subcutaneously on the left back in a dose of 0.1 ml/mouse, and 0.1 ml/mouse of 14C-4-NQO (10 mg/10 μCi/ml, as egg lecithin suspension) was injected subcutaneously on the right back at the same time.

Mice were killed by cervical dislocation 30 min after the administration, ice-cold 1.15% KCl-0.01M phosphate buffer (pH 7.4) was injected directly into the heart to remove blood in the lung and liver, and the lung and liver were rapidly removed. The organs were washed with ice-cold 1.15% KCl-0.01M phosphate buffer (pH 7.4), and blood and moisture were wiped off. Each organ was minced finely, and homogenized with the same buffer to make a 25% (w/v) homogenate by a glass homogenizer.

To this homogenate, 5-fold volume of benzene was added to extract unchanged 14C-4-NQO, and centrifuged at 3000 rpm for 10 min. A definite volume of the benzene layer was injected subcutaneously on the left back in a dose of 0.1 ml/mouse, and 0.1 ml/mouse of 14C-4-NQO (10 mg/10 μCi/ml, as egg lecithin suspension) was injected subcutaneously on the right back at the same time.

The aqueous phase separated from the benzene layer was mixed with 0.2 ml of 6N HCl and 3.0 ml of CHCl₃, the mixture was centrifuged at 3000 rpm for 10 min, and 30 μl of its supernatant was applied in a band on the thin-layer plate (silica gel 60F, 0.25 mm thickness, Merck). The plate was developed with a solvent system of sec-BuOH:AcOEt:H₂O (2:1:1, v/v), and bands corresponding to each of the metabolites (Rf values; 4-HAQO 0.60, 4-AQO 0.35, 4-OHQO 0.72, 4-AQ 0.16, 4-OHQ 0.84, unknown 0.05) were each scraped off from the plate. 3.0 ml of methanol was added to each, and left at room temperature for 30 min. The methanol extract was centrifuged at 3000 rpm for 10 min. 15.0 ml of a scintillation fluid, a Triton-phosphor, was added to 1.0 ml of the supernatant, and radioactivity of each metabolite was measured with a liquid scintillation spectrometer.

**RESULTS**

**Activity of 4-NQO Reductase** Effect of subcutaneous administration of aluminium chloride on the activity of 4-NQO reductase in the lung and liver was examined and its result is given in Table I. After administration of 125 μg/mouse of aluminium chloride, 4-NQO reductase activity in the lung was 136% (P<0.05) and that in the liver was 152% (P<0.01) of those of the control. In the mice given 500 μg/mouse of aluminium chloride, the reductase activity in the lung was 166% (P<0.01) and that in the liver was 168% (P<0.01) of those of the control. Effect of aluminium chloride on 4-NQO reductase activity appeared slightly stronger in the group given 500 μg/mouse of aluminium chloride, both in the lung and liver.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AlCl₃ (μg/mouse)</th>
<th>Activity a)</th>
<th>% of control</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Lung</td>
<td>—</td>
<td>16.5±1.09</td>
<td>100</td>
<td></td>
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<tr>
<td></td>
<td>125</td>
<td>22.5±1.88</td>
<td>136</td>
<td>&lt;0.05</td>
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<tr>
<td></td>
<td>500</td>
<td>27.4±2.41</td>
<td>166</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>—</td>
<td>37.9±3.38</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>57.5±3.70</td>
<td>152</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>63.6±4.54</td>
<td>168</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

a) Expressed as μg products/mg protein/30 min. Six mice were used for each group and each value is their mean±SE. Animals were sacrificed 2 hr after the subcutaneous injection of AlCl₃.

Table I. Effect of Aluminium Chloride on the Activity of 4-Nitroquinoline 1-Oxide Reductase in the Lung and Liver of Mice Following Subcutaneous Injection of AlCl₃

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Table II. Effect of Aluminium Chloride on the Activity of 4-Hydroxyaminoquinoline 1-Oxide Reductase in the Lung and Liver of Mice Following Subcutaneous Injection of AlCl₃

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AlCl₃ (µg/mouse)</th>
<th>Activity a)</th>
<th>% of control</th>
<th>P</th>
</tr>
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<tr>
<td>Lung</td>
<td>—</td>
<td>15.3±0.82</td>
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<tr>
<td></td>
<td>125</td>
<td>24.2±1.44</td>
<td>158</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>500</td>
<td>24.8±1.27</td>
<td>162</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Liver</td>
<td>—</td>
<td>95.4±8.01</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>149.1±6.24</td>
<td>156</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>149.9±4.10</td>
<td>157</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

a) Expressed as µg of 4-aminoquinoline 1-oxide formed. Six mice were used for each group and each value is their mean ± SE. Animals were sacrificed 2 hr after the subcutaneous injection of AlCl₃.

Activity of 4-HAQO Reductase Effect of subcutaneous administration of aluminium chloride on the activity of 4-HAQO reductase in the mouse lung and liver is summarized in Table II. Subcutaneous administration of 125 or 500 µg/mouse of aluminium chloride resulted in increased enzyme activity in the lung, being 158% (P<0.01) and 162% (P<0.01) of the control, respectively. The enzyme activity in the liver was 156% (P<0.01) and 157% (P<0.01) of the control, respectively. Thus, 4-HAQO reductase activity in the lung and liver was elevated by the subcutaneous administration of aluminium chloride but the elevation was not affected by the dose of aluminium chloride given.

Distribution of 14C-4-NQO and Its Metabolites in the Lung and Liver Mice were injected subcutaneously with 500 µg/mouse of aluminium chloride on the left back and 1 mg/1 µCi/mouse of 14C-4-NQO on the right back at the same time, and radioactivities in the lung and liver 0.5, 1, 2, and 4 hr after the administration were measured. Its result is illustrated in Fig. 1.

Simultaneous subcutaneous administration of aluminium chloride and 14C-4-NQO resulted in significantly decreased radioactivity in the lung 0.5 and 1 hr after the administration, compared with those of the control, while the radioactivity in the liver was inversely higher than that of the control. At 2 hr after the administration, radioactivities in the lung and liver were not different from those of the control.
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Fig. 2 shows the distribution of $^{14}$C-4-NQO and its metabolites in the lung and liver 0.5 hr after the subcutaneous administration of aluminium chloride and $^{14}$C-4-NQO. Simultaneous administration of aluminium chloride and $^{14}$C-4-NQO reduced the distribution of 4-NQO, 4-HAQO, and unknown substance(s) in the lung, the rate being 55%, 61%, and 74%, respectively, of those of the control. On the other hand, distribution of 4-AQO and 4-OHQO in the lung was increased, being 129% and 139%, respectively, of those of the control. There was no difference in the distribution of 4-AQ and 4-OHQ in the lung from that of the control.

In the liver, distribution of 4-NQO and 4-HAQO was respectively 80% and 84% of those of the control, and this difference was not statistically significant but the tissue distribution of other metabolites, 4-AQO, 4-OHQ, and unknown substance(s) was increased statistically to 152%, 144%, and 150%, respectively, of those of the control. Distribution of 4-AQ in the liver was not different from that of the control, same as in the lung.

DISCUSSION

Effect of subcutaneous administration of aluminium chloride on the activities of 4-NQO reductase and 4-HAQO reductase, and the organ distribution of carcinogenic substance(s) were examined by using mouse lung, which is the target organ of 4-NQO carcinogenesis, and the liver, which is one of important organs in drug metabolism.

Subcutaneous administration of aluminium chloride to mouse results in significant

Radioactivity per tissue (10^4 cpm)

![Bar graph showing tissue distribution of 4-NQO and its metabolites in the lung and liver of mice after simultaneous subcutaneous injection of $^{14}$C-4-NQO and AlCl₃]

Fig. 2. Effect of aluminium chloride on tissue distribution of $^{14}$C-4-NQO and its metabolites in the lung and liver of mice after simultaneous subcutaneous injection of $^{14}$C-4-NQO and AlCl₃

a) $R_0$ 0.05. $^{14}$C-4-NQO (1 mg/l μCi/mouse) injected subcutaneously. $^{14}$C-4-NQO and AlCl₃ (500 μg/mouse) injected subcutaneously at the same time. Animals were sacrificed 0.5 hr after the injection. Six mice were used for each group and each value is their mean±SD. Numbers in parentheses represent percentage to the control.
elevation of 4-NQO and 4-HAQO reductase activities in the lung and liver, compared with the control. It is still not clear whether such elevation of these reductase activities by the subcutaneous administration of aluminium chloride is due to the binding of aluminium to nucleic acids,5,11) thereby increasing the template activity and increasing biosynthesis of RNA,1) or whether aluminium plays the role of an activator for these reductases. These points require further elucidation.

Simultaneous subcutaneous administration of $^{14}$C-4-NQO and aluminium chloride on either side of mouse back results in lower radioactivity in the lung 0.5 and 1 hr after the administration, compared with the control, while the radioactivity in the liver was higher. At 2 hr after the administration, radioactivity in the lung and liver were not different from those of the control. This fact suggested that aluminium chloride gives some kind of an effect on the tissue distribution of 4-NQO within 2 hr after its administration.

Further examination was made on the distribution of $^{14}$C-4-NQO and its metabolites in the lung and liver 0.5 hr after the simultaneous subcutaneous administration of $^{14}$C-4-NQO and aluminium chloride. Distribution of carcinogenic 4-NQO and 4-HAQO in the lung was reduced significantly compared with that of the control by the simultaneous administration of aluminium chloride, and the distribution of their metabolites, noncarcinogenic 4-AQO and 4-OHQ, was increased. Decrease of the radioactivity in the lung by the simultaneous subcutaneous administration of $^{14}$C-4-NQO and aluminium chloride seemed to be the decrease of 4-NQO, 4-HAQO, and unknown substance(s). Consequently, decrease of 4-NQO and 4-HAQO in the lung by the subcutaneous administration of aluminium chloride is assumed to be one of the factors in the mechanism of suppression of carcinogenesis by aluminium chloride.

On the other hand, distribution of 4-NQO and 4-HAQO in the liver after simultaneous administration of $^{14}$C-4-NQO and aluminium chloride was not different from that of the control but the distribution of other metabolites, 4-AQO, 4-OHQ, and unknown substance(s), was increased compared with that of the control.

Even if unknown substance(s) was not merely a polar metabolite but an ultimate carcinogen, simultaneous administration of aluminium chloride has decreased the distribution of unknown substance(s) in the lung, the target organ of 4-NQO carcinogenesis, so that, with the decrease in the distribution of 4-NQO and 4-HAQO in the lung, decrease of unknown substance(s) may be assumed as one of the factors for the mechanism of carcinogenesis suppression by aluminium chloride.

If the distribution and accumulation of a carcinogenic substance(s) in the target organ were to be taken as the first step of carcinogenic process, then the decrease in the distribution of carcinogenic 4-NQO and 4-HAQO in the lung by the subcutaneous administration of aluminium chloride would constitute one of the factors responsible for the suppression of carcinogenesis by aluminium chloride.

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References

6) Mori, K., Gann, 53, 303~308 (1962).
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