EFFECTS OF GLYCEROL ON 4-NITROQUINOLINE 1-OXIDE INDUCED PULMONARY TUMORIGENESIS IN ddY MICE

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The effects of glycerol administration on pulmonary tumorigenesis induced by 4-nitroquinoline 1-oxide (4NQO) were examined in male ddY mice, that were given 5% glycerol solution instead of drinking water after a subcutaneous injection of 4NQO. The incidence of pulmonary tumor-bearing mice and the mean number of induced tumors per mouse were significantly enhanced in mice given glycerol after 4NQO treatment, compared with mice given 4NQO alone. The results demonstrate effects including that of promotion by glycerol in 4NQO-induced pulmonary tumorigenesis.

Key word: Tumor promoter — Pulmonary tumorigenesis — Clara cell — Glycerol — 4-Nitroquinoline 1-oxide

Several tumor promoters or modifiers related to various carcinogenic processes are known. In the field of pulmonary tumorigenesis, however, only a few promoters have been reported, such as butylated hydroxytoluene (BHT),13 bleomycin,20 and isoproterenol (unpublished results).

In pulmonary tumorigenesis, we demonstrated that the bronchiolar non-ciliated (Clara) cell is a candidate as the metabolic activating cell of certain respiratory carcinogens and is a possible progenitor cell of peripheral carcinoma in the lung.5,6) The exact function of the Clara cell has long been unknown, but recent studies have revealed possible participation in the production of pulmonary surfactant,5,6) high lipid metabolism,5,6) and metabolism related to xenobiotics.7-11)

In preliminary studies, we demonstrated that a 2-week oral administration of glycerol induced significant morphological changes (such as hyperplasia of the smooth endoplasmic reticulum) selectively in the Clara cells, suggesting some alterations in metabolic activity (unpublished results). If the metabolic function of Clara cells is activated by glycerol, administration of glycerol may modify the incidence of pulmonary tumors induced by a carcinogen. However, it is still unknown whether such metabolic alterations of Clara cells result in an increase of cancer incidence, since both detoxication and activation of carcinogens in the Clara cells might be enhanced by the modifiers of Clara cell function. The present study, therefore, was performed to examine the effect of
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The animals used were 6-week-old male ddY mice (Shizuoka Laboratory Animal Center, Shizuoka). 4NQO, 0.3 mg/mouse, was administered as a single subcutaneous injection in a mixture of olive oil and cholesterol (20:1) according to Mori’s method at the beginning of experimental week 1 (1NQ). A 5% glycerol solution was given as drinking water ad libitum. The details of the experimental design are shown in Fig. 1.

All mice were sacrificed at 25 weeks after the 4NQO administration. At autopsy, the lungs, fixed by intratracheal instillation of 1% glutaraldehyde, were separated into each lobe and the number of induced tumors was counted using a dissecting microscope. Paraffin-embedded lung tissues were semi-serially sectioned and stained with hematoxylin and eosin to confirm the number of tumors histologically.

The results are summarized in Table I: 80% (1NQ+1G4) and 88% (1NQ+1G25) of mice that received 4NQO on the first day and glycerol from the beginning of the experiment had pulmonary tumors. The control groups, NT, 1G25 and 1NQ showed 0, 0 and 10% incidences, respectively. The mice receiving glycerol treatment for 21 weeks following a 4-week intermission after 4NQO injection (1NQ+5G25) showed 70% tumor incidence.

The mean number of pulmonary tumors per mouse showed the same tendency as that shown by the tumor incidence, i.e., 3.5 and 2.3 in the groups given combined administration of 4NQO and glycerol from the beginning of the experiment, and 1.9 in the mice treated with glycerol following a 4-week intermission after 4NQO injection. In the control groups, no tumors were observed except in 4NQO-treated mice, the number being 0.1 per mouse. Histologically all the pulmonary tumors showed adenomatous growth. There was no obvious morphological difference among the groups.

As shown in Table I, all the groups receiving 4NQO and subsequent glycerol treatment (1NQ+1G4, 1NQ+1G25, 1NQ+5G25) showed a statistical increase in both tumor incidence (P<0.001, P<0.001, P<0.05) and mean tumor count (P<0.05, P<0.01, P<0.05) compared with the mice given 4NQO alone. There was no difference statistically among the groups receiving combined administration of 4NQO and glycerol. This enhancing effect of glycerol on 4NQO-carcinogenesis can be considered to be a promoting action. However, glycerol treatment for 4 weeks (1NQ+1G4) seems rather short for promotion. Thus, the apparent effective promotion by glycerol treatment for 4 weeks was unexpected and may require further studies to investigate whether the effects of glycerol treatment for 4 weeks and 25 weeks (1NQ+5G25) are due to the same mechanism.

As mentioned above, glycerol seems to be a metabolic activator of Clara cells, and therefore glycerol treatment prior to 4NQO injection may also modify 4NQO-tumorogenesis. The function of Clara cells altered by glycerol may increase or even decrease the degree of tumorigenesis. Therefore, it seems to be necessary to examine the ef-

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of evaluable mice</th>
<th>No. of tumor-bearing mice</th>
<th>Incidence of tumor-bearing mice (%)</th>
<th>Total tumor number</th>
<th>Mean tumor number per mouse (m±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
</tr>
<tr>
<td>1G25</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
</tr>
<tr>
<td>1NQ</td>
<td>10</td>
<td>1</td>
<td>80±4</td>
<td>35</td>
<td>3.5±4.40</td>
</tr>
<tr>
<td>1NQ+1G4</td>
<td>10</td>
<td>8</td>
<td>88±4</td>
<td>21</td>
<td>2.3±1.540</td>
</tr>
<tr>
<td>1NQ+1G25</td>
<td>9</td>
<td>8</td>
<td>70±4</td>
<td>19</td>
<td>1.9±2.060</td>
</tr>
</tbody>
</table>

a) and b) are significantly different from 1NQ at P<0.001 and P<0.05, respectively.
b) and d) are significantly different from 1NQ at P<0.05 and P<0.01, respectively.
effect of pretreatment with glycerol. Moreover, the promoting effect of glycerol found in the present study should be confirmed in other in vivo pulmonary carcinogenesis systems. In vitro studies may also be beneficial to clarify the mechanism of this effect of glycerol.

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REFERENCES