Pretreatment with 8-Methoxypsoralen, a Potent Human CYP2A6 Inhibitor, Strongly Inhibits Lung Tumorigenesis Induced by 4-(Methylnitrosamo)-1-(3-Pyridyl)-1-Butanone in Female A/J Mice

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Abstract

Human CYP2A6 has been recognized as being involved in the mutagenic activation of promutagens such as the tobacco-specific nitrosamine, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanone (NNK). Methoxsalen (8-methoxypsoralen) was reported to inhibit CYP2A6. In the present study, the inhibitory effects of methoxsalen on NNK-induced lung tumorigenesis in female A/J mice were examined. Female A/J mice were treated with methoxsalen at doses of 50 or 12.5 mg/kg body weight, given by stomach tube, daily for 3 days. One h after the final treatment, NNK was injected i.p. at a dose of 2 mg/mouse. The experiments were terminated 16 weeks after the first methoxsalen treatment, and lung adenomas were analyzed. Pretreatment of methoxsalen significantly reduced tumor incidence from 93.8% to 16.7% (50 mg/kg) and 20.0% (12.5 mg/kg), and tumor multiplicity from 5.97 to 0.23 (50 mg/kg) and 0.25 (12.5 mg/kg) tumors/mouse. These results clearly demonstrated that methoxsalen, a potent human CYP2A6 inhibitor, is a strong chemopreventive agent against NNK-induction of lung tumorigenesis.

Introduction

Lung cancer is one of the most common cancers in the world, and cigarette smoking is regarded as the overwhelming cause. In our previous study, Japanese male smokers with CYP2A6* gene deletion-type polymorphism were shown to have a reduced lung cancer risk in a hospital-based case control study (1). Furthermore, it was demonstrated that CYP2A6* gene deletion reduces oral cancer risk in betel quid chewers in Sri Lanka (2). CYP2A6 is in fact recognized to be involved in the mutagenic activation of promutagens such as tobacco-specific N-nitrosamines (3). NNK, one such tobacco-specific N-nitrosamine, conceivably plays an important role in tobacco-related human lung cancer, given its strong potential to induce lung tumorigenesis in rodents (4).

CYP2A6 is known to contribute to coumarin 7-hydroxylation (5). In addition, it has been shown to be the predominant catalyst of human liver microsomal C-oxidation of nicotine (6). A genetic polymorphism of CYP2A6 is recognized as one of the causes of interindividual differences in metabolism of coumarin. We examined previously the capacity of organosulfur compounds to inhibit the coumarin 7-hydroxylase activity of human CYP2A6 (7). Among the series tested, 4,4′-dipyridyl disulfide was the most potent inhibitor of CYP2A6, followed by 4,4′-dipyridyl sulfide. Methoxsalen is also reported to inhibit CYP2A6 (8, 9). In mutagenicity testing using Salmonella typhimurium YG7108 expressing high levels of CYP2A6, it was found that both methoxsalen and 4,4′-dipyridyl sulfide at low concentrations inhibited mutagenic activity of NNK (10).

If one of the causes of human lung cancer is dependent on metabolic activation of a tobacco-specific N-nitrosamine, inhibition of CYP2A6 by chemicals may result in chemoprevention of tobacco-related lung cancer. Therefore, in the present study, we examined the potential inhibitory effects of methoxsalen on NNK-induced lung tumorigenesis in female A/J mice.

Materials and Methods

Chemicals. Methoxsalen was purchased from Sigma (St. Louis, MO) and NNK from Toronto Research Chemicals (Toronto, Ontario, Canada).

Animals. Female A/J mice (5 weeks of age), purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan), were maintained in the Kagawa Medical University Animal Facility according to the institutional animal care guidelines. All of the animals were housed in polycarbonate cages with white wood chips for bedding, and given free access to drinking water and a basal diet, Oriental MF (Oriental Yeast Co., Ltd., Tokyo, Japan), under controlled conditions of humidity (60 ± 10%), lighting (12 h light/dark cycle), and temperature (24 ± 2°C).

Experimental Design. Experiment 1. When the mice were 7 weeks of age, they were pretreated with methoxsalen (50 mg/kg body weight in 0.2 ml corn oil, given by stomach tube) or an equal volume of corn oil (vehicle control) daily for 3 days. One h after the final treatment, each group was given a single dose of NNK (2 mg/0.1 ml saline/mouse, i.p.) or an equal volume of saline (vehicle control). They were then maintained without additional treatment. The experiment was terminated 16 weeks after the first methoxsalen treatment. All of the surviving mice were killed under ether anesthesia. At autopsy, their lungs were excised and weighed, infused with 10% neutral buffered formalin, and carefully inspected grossly. All of the macroscopically detected lung nodules were counted, and each lung lobe was examined histopathologically. Lung lesions, hyperplasias, and adenomas were diagnosed according to the criteria of “Tumors of the mouse” (11), and the number of adenomas was counted under a microscope. Hyperplasias were not evaluated in these experiments.

Experiment 2. The second experiment was conducted with the same design to determine the dose-response relationship with methoxsalen administered at 50 or 12.5 mg/kg body weight (groups 1a and 1b). Methoxsalen and NNK were given by the same routes and the same solvents as in experiment 1. An additional group was the vehicle control alone, treated with corn oil (given by stomach tube) and saline (i.p.).

Statistical Analysis. The incidences of lung proliferative lesions were analyzed by Fisher’s exact probability test and data for multiplicity by Student’s t test.
Results

Experiment 1. Lung whitish nodules were readily detected in the NNK alone group macroscopically but were very rare in the methoxsalen + NNK group (Fig. 1). Lung lesions carcinoma could not be detected in any of the animals. Incidences and multiplicities of lung adenomas (Fig. 2) in experiment 1 are summarized in Table 1. Pretreatment of methoxsalen significantly reduced both incidences and multiplicities of lung adenomas (group 1).

Experiment 2. Because strong inhibition of lung tumorigenesis by methoxsalen was observed in the first experiment, a second study was performed to confirm inhibitory effects, and also to analyze the dose dependence. Treatment with both 50 and 12.5 mg/kg significantly reduced incidences and multiplicities of lung adenomas (group 1).

The results of experiments 1 and 2 are summarized in Table 1. The incidences and multiplicities in methoxsalen-treated groups (groups 1a, 1b) were significantly more reduced than those values in the NNK alone group ($P < 0.001$). Even with NNK-untreated mice (groups 2 and 4), incidences of lung adenomas showed a tendency to decrease from 28.6 to 4.6% on methoxsalen treatment, but this did not reach statistical significance ($P = 0.0637$). Multiplicities of adenomas also showed a tendency to decrease by methoxsalen treatment ($P = 0.0505$).

Discussion

The present study demonstrated that pretreatment of methoxsalen dramatically inhibits NNK-induced lung tumorigenesis in female A/J mice. With this carcinogen, the initial event is reported to be the formation of O6-methylguanine, a major promutagenic adduct that leads to GC→AT transitional mispairing and subsequent activation of the K-ras proto-oncogene (12, 13). These events are dependent on metabolic activation of NNK. The first activation step of NNK is thought to be hydroxylation of the carbon atom located at the 6-position of the N-nitroso group, catalyzed by cytochrome P450 (14). The subfamilies of cytochrome P450 mainly involved in hydroxylation of nitrosamines are thought to be CYP2A in humans and rodents (15, 16).

CYP2A6 catalyzes coumarin 7-hydroxylation (5) and nicotine metabolism (17), and is responsible for the metabolic activation of N-alkylnitrosamines, including NNK (16). We established recently a S. typhimurium YG7108 coexpressing CYP2A6 and human NADPH-cytochrome P450 reductase. Using this model, we demonstrated that CYP2A6 is involved in the mutagenic activation of tobacco-related N-nitrosamines such as NNK (18). In the present

Table 1 Data for experiment 1 and experiment 2: incidences and tumors/mouse of NNK-induced lung adenoma in A/J mice treated with methoxsalen

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 1 and experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence (%)</td>
<td>Tumors/mouse</td>
<td>Incidence (%)</td>
<td>Tumors/mouse</td>
</tr>
<tr>
<td>1a</td>
<td>Methox-50 + NNK</td>
<td>0/10 (0%)</td>
<td>20</td>
<td>0.35 ± 0.75%</td>
</tr>
<tr>
<td>1b</td>
<td>Methox-12.5 + NNK</td>
<td>ND</td>
<td>20</td>
<td>0.25 ± 0.55%</td>
</tr>
<tr>
<td>2</td>
<td>Methox-50</td>
<td>1/11 (9.1)</td>
<td>11</td>
<td>0.09 ± 0.30</td>
</tr>
<tr>
<td>3</td>
<td>NNK</td>
<td>15/15 (93.3)</td>
<td>17</td>
<td>3.29 ± 2.34</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle control</td>
<td>ND</td>
<td>14</td>
<td>0.43 ± 0.85</td>
</tr>
</tbody>
</table>

* Number of mice examined.
* Number of mice observed each lesion (%).
* Mean ± SD.
* Significantly different from group 3 by Fisher’s exact probability test ($P < 0.001$).
* Significantly different from group 3 by Student’s t test ($P < 0.001$).
* ND, not determined.
study, it is, thus, probable that inhibitory effects of methoxsalen were due to inhibition of the metabolic activation of NNK via CYP2A6.

Methoxsalen in fact is reported to inhibit CYP2A6 (8, 9) and mouse CYP2A5-mediated coumarin 7-hydroxylation (19). Furthermore, high concentrations of methoxsalen have been reported to also inhibit CYP2C9 (20), CYP1A2, CYP2B6, CYP3A4, and CYP3A5 activities (8). It has been shown that methoxsalen is a substrate for CYP2A6 and that enzyme inhibition is due to competitive interactions (9, 21). The metabolism-dependent inactivation of CYP2A6 by methoxsalen occurs at low concentrations and a high rate (21). In a mutagenicity test using S. typhimurium YG7108 expressing high levels of CYP2A, very low concentrations of methoxsalen significantly inhibited metabolic activation of NNK (10). In the present study, a low dose (12.5 mg/kg) of methoxsalen inhibited lung tumorigenesis to the same extent as the high dose (50 mg/kg).

In conclusion, the results of this study indicate that methoxsalen, a potent human CYP2A6 inhibitor, is a strong chemopreventive agent for NNK-induced lung tumorigenesis. These data point to a feasibility of CYP2A6 inhibitors as possible chemopreventive agents for nicotine-related cancer. Additional studies are now ongoing in our group, for example to determine mRNA expression levels of mouse orthologous form(s) of human CYP2A6 and metabolism of NNK in methoxsalen-treated mice.

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References

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