Abstract. Background/Aim: Research has shown that chemoprevention may be effective against the development of lung cancer. The purpose of the present study was to evaluate the effect of oral chemoprevention in a mouse model of tobacco carcinogen-induced lung tumor. Materials and Methods: A total of 60 A/J mice were randomized to a normal diet, a diet with low calcium, or a chemoprevention diet with acetylsalicylic acid, 1-α 25(0H)2-vitamin D3 and calcium. In addition to the diet, mice received carcinogens by oral gavage for ten weeks. Results: The chemoprevention diet significantly reduced the number of animals with tumors [1 vs. 13, (p<0.001)] and the median number (range) of tumors [0 (0-1) vs. 1 (0-4), (p<0.001)] compared to controls. No signs of toxicity in relation to the diets were observed. Conclusion: The chemoprevention diet had a protective effect against tumor development in the mouse lungs.

Materials and Methods

Animals. In 60 A/J mice (Charles River laboratories, Kungsbacka, Sweden) with specific pathogen-free (SPF) status, lung tumors were induced by tobacco carcinogens as previously described (11). The animals were caged in polypropylene cages in a negative pressure isolator equipped with HEPA filters (class EU10). A light-cycle of 12 hours' light/dark and a temperature of between 18˚ and 22˚C were maintained. Drinking water was UV-irradiated for sterilization. The animals were handled by educated personnel with veterinary supervision and welfare for each animal was evaluated on a daily basis. These animals were also used to evaluate the chemoprevention regimen to prevent bladder tumors, and the weight, water and diet consumption of the animals have been previously reported (12). Prior to initiation, the study was approved by the Danish agency for animal experiments (license number 1997-101-27).

Diet and chemoprevention. Mice were randomized to one of the three diets. Group A (control group) received a normal diet containing 5,000 μg/g calcium; group B (low-calcium group) received a diet containing 2,500 μg/g calcium; and group C (intervention group) received a diet containing 300 μg/g ASA, 0.02 μg/kg 1-α 25(0H)2-vitamin D3 (vitamin D) and 7,500 μg/g calcium.

After two weeks feeding on the respective diets ad libitum, tumor induction was initiated with carcinogens administered orally. The precise dosages of the chemopreventive drugs were decided on the basis of previous pilot studies where both toxicology and efficacy were tested (13). The pilot studies discovered that a dosage lower than 300 μg/g ASA had no effect and a dosage higher than 0.02 μg/kg vitamin D3 resulted in kidney damage to the animals.
Induction of tumors. Benzo[a]pyrene (Sigma-Aldrich, Brøndby, Denmark) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Chemos GmbH, Regenstauf, Germany) were administered with oral gavage at a dosage of 3 μmol per mouse for each substance with cotton oil as a vehicle as described elsewhere (11). All mice received both carcinogens, which were administered once a week for a total of ten weeks.

Evaluation of effect. The experiment was terminated two weeks after the last carcinogen administration. The lung tissue of each animal was dissected and the number of tumors was counted. Body weight, diet consumption and drinking water consumption were recorded for each animal once a week for the entire experiment. Tissues from random tumor samples was evaluated for histology to confirm the presence of adenoma. This experiment was designed as a hypothesis-testing study. Hence, no evaluation of the biochemical pathways responsible for any effect was performed.

Statistics. To evaluate the distribution of deaths between the groups, Chi-square test was used. The Fischer’s exact test was used to compare the presence (yes/no) of tumors between the diet groups. The Mann-Whitney test was used to compare the number of tumors between the groups and Friedman test was used to determine differences over time between the groups for weight, diet and water consumption data, since these data were not normally distributed. Data in figures are presented as median values. A \( p \)-value below 0.05 was considered statistically significant.

Results

The number of deaths was comparable in the three groups (Table I).

<table>
<thead>
<tr>
<th>Group A (control)</th>
<th>Group B (low calcium)</th>
<th>Group C (intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of deaths</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No. of animals with tumors</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>No. of tumors in the animals, median (range)</td>
<td>1 (0-4)</td>
<td>0 (0-2)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Compared with group A, \textsuperscript{b}compared with group B, significant results are shown in bold.

Development of tumors. There were significantly fewer animals with tumors in the intervention group (group C) compared with the control group (\( p<0.001 \)). Moreover, the number of tumors was significantly lower in the intervention group compared with controls (\( p<0.001 \)) (Table I).

The number of animals with tumors was significantly lower in the intervention group compared with the low-calcium group (\( p=0.041 \)). The number of tumors in these animals was also lower, however, without reaching statistical significance (\( p=0.085 \)) (Table I).

Weight gain and consumption of diet and water. The weight gain during the treatment period was different between group A and group C (\( p=0.019 \)), which may be due to a higher initial weight for animals in group C. The median weights were comparable throughout the remaining part of the experiment (Figure 1). The weight gain was comparable between group B and group C (\( p=0.414 \)).

The water consumption was different between group A and group C (\( p=0.049 \)), which may result from a higher initial consumption in group C. The consumption was comparable between group B and group C (\( p=0.082 \)) (Figure 2).

The diet consumption was comparable between the three groups (group A \( vs. \) group C (\( p=0.306 \)), group B \( vs. \) group C (\( p=0.447 \)) (Figure 3).

Histological evaluation. Tissue samples confirmed that all analyzed tumors were adenomas.

Discussion

In this study, we found that chemoprevention with a novel combination of ASA, vitamin D and calcium had a preventive effect on the number of tumors and the number of animals with tumors. Based on the results for weight, diet and water consumption, the chemoprevention treatment showed complete absence of toxic effects.

NSAIDs may be effective against lung cancer development due to inhibition of the cyclooxygenase (COX)-2 enzyme. Studies have shown that this enzyme is expressed in human non-small cell lung cancer, and both the COX-1 and COX-2 enzymes are expressed in lung tumors in mice (14, 15). Previous research has shown an effect of NSAIDs, including ASA, on experimentally-induced lung tumors in mice (3-5). Vitamin D has anti-proliferative and pro-apoptotic properties and studies have suggested that this vitamin may protect against different types of cancers (6, 16), including lung cancer (17). Vitamin D in combination with calcium has been shown to be protective against lung cancer in an observational study (9). Calcium alone was effective as chemoprevention for colorectal cancer, and in...
particular colorectal adenomas, which is thought to be due to its involvement in the regulation of various genes and cellular pathways (18, 19). However, the evidence that calcium alone may be protective against lung cancer is still scarce (20).

A limitation of this study was that the affected biochemical pathways were not investigated. Moreover, this study did not evaluate the tested agents both as monotherapy and in combination. Thus, the agent(s) responsible for the effect are unknown. The effect in the intervention group was less pronounced in comparison with the low-calcium group when compared with the control group. This may imply that a low calcium level in the diet is favorable over a high level. However, whether a lower level of calcium in the diet of the intervention group would have created a greater effect remains unknown.

Results from the present study indicate that a combination therapy might be suited as chemoprevention in high-risk patients, such as smokers and ex-smokers. However, prior to clinical evaluation, a study investigating which drugs are responsible for the effect should be initiated. Furthermore, it should be considered if this combination treatment has long-term adverse effects in humans, e.g. gastrointestinal bleeding (10).
In conclusion, we found a chemopreventive effect of the combination of ASA, vitamin D and calcium against tobacco carcinogen-induced lung tumors in mice. The effect of these drugs alone and in combination should be evaluated together with biochemical analyses, in order to determine the compounds and pathways responsible for the effect.

Declaration of Interest

The Author declare that they have no conflicts of interest or financial interest associated with the study and no financial interest in the patents cited in reference 13.

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References


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