Advances in Brief

Chemopreventive Effects of myo-Inositol and Dexamethasone on Benzo[a]pyrene and 4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone-induced Pulmonary Carcinogenesis in Female A/J Mice

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Abstract

The objective of the present investigation was to prevent cancer of the lung by use of chemopreventive agents. Administrations of diets containing added myo-inositol or dexamethasone singly or in combination (the latter being the most potent) are being studied for this purpose. In previous work, the two compounds were shown to inhibit benzo(a)pyrene [B(a)P]-induced pulmonary adenoma formation in female A/J mice when fed during the postinitiation period [i.e., starting 1 week after the last of three administrations of B(a)P by oral intubation]. In the present investigation, a longer administration schedule was used, which encompasses both the initiation and the postinitiation stages of carcinogenesis. The feeding of the test compounds was started 2 weeks prior to the first dose of carcinogen and continued for the duration of the experiment. Under these conditions, reductions in tumor formation were: myo-inositol, 64%; dexamethasone, 56%; and both together, 71% (P < 0.001 for all three). Addition of both compounds resulted in the largest inhibition that has been achieved with this experimental model as used in these investigations. Studies have begun of inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary adenoma formation by myo-inositol and dexamethasone. The two compounds inhibit pulmonary carcinogenesis when fed singly or in combination. When fed throughout the entire protocol, reductions in tumor formation were: myo-inositol, 64%; dexamethasone, 41%; and both together, 71% (P < 0.001 for all three). The results of these investigations demonstrate that myo-inositol and dexamethasone inhibit pulmonary adenoma formation resulting from exposures to two major pulmonary carcinogens, B(a)P and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

Introduction

The present investigation is part of a continuing effort to develop effective chemoprevention of carcinogenesis of the lungs. In previous studies, addition of myo-inositol and dexamethasone to the diet singly or in combination (the latter being the most potent) was found to reduce B(a)P-induced pulmonary adenoma formation when the diet was fed during the postinitiation period (1). There is a great need for compounds that can inhibit pulmonary neoplasia when given during the postinitiation period of the carcinogenic process. Few chemopreventive agents have been shown to have this property (2, 3). The first demonstration of the chemopreventive effects of myo-inositol was in carcinogenesis of the large bowel. In this work, myo-inositol and inositol hexaphosphate (phytate) were both found to prevent carcinogen-induced neoplasia of this organ site when administered in the postinitiation period. Most of the experiments were done using inositol hexaphosphate (4–6). This compound is a common constituent of a large number of foods of plant origin (7), in which myo-inositol can also be present. In addition, myo-inositol can be formed within the intestinal tract as a result of hydrolysis of inositol hexaphosphate by the enzyme phytase, which occurs in the intestinal mucosa (8). Much of the ingested inositol hexaphosphate is hydrolyzed to inositol (8). The use of inositol hexaphosphate as a chemopreventive agent presents a problem in that it is a chelating agent (9). myo-Inositol does not have this property. It has exceedingly little toxicity, which makes it an attractive compound for study. It has been administered to humans and animals in high doses without producing adverse reactions (1, 10, 11). myo-Inositol has been shown to increase pulmonary surfactant synthesis when administered to immature animals (12). Other than this, very little information exists as to any effects it has on the lung. The mechanism(s) by which it inhibits carcinogenesis is not known.

The second compound under investigation is dexamethasone, a synthetic glucocorticoid. This compound has been shown to inhibit carcinogenesis of the skin, forestomach, and lungs of the mouse when given in the postinitiation period (13, 14). In initiation/promotion experiments of epidermal carcinogenesis of the mouse, dexamethasone as well as other glucocorticoids have been found to be highly effective inhibitors when administered in the promotion phase of the study (13, 14). Dexamethasone has also been found to inhibit B(a)P-induced pulmonary adenoma formation and squamous cell carcinogenesis of the forestomach in female A/J mice when given in the postinitiation period (1). Dexamethasone has a large number of biological effects, including the capacity to mature type 2 alveolar cells, the major cell type occurring in pulmonary tumors in the experimental model used in the present study (15–19). Which effect or combination of effects is responsible for the cancer prevention properties of dexamethasone has not been established.

In earlier studies, both myo-inositol and dexamethasone were shown to inhibit pulmonary adenoma formation in female A/J mice when administered in the postinitiation period (1). The selection of this period of the carcinogenic process for study was based on data demonstrating this attribute in other tissues as described above. In the present investigation, the effects of the two compounds administered during an earlier period of the carcinogenic process (i.e., starting 2 weeks prior to the first administration of carcinogen and continuing until 7 days after the last dose) have been studied. In addition, experiments have been carried out in which the compounds were fed throughout the entire course of the experiment. The prior experiments with myo-inositol and dexamethasone as inhibitors of pulmonary tumor formation were limited to those in which B(a)P was used as the carcinogen. In the experimental work presented here, the capacity of the two compounds to prevent pulmonary adenoma formation resulting from the administration of a second important pulmonary carcinogen, NNK, has been determined (20, 21).
CHEMOPREVENTION OF LUNG CANCER IN AJ MICE

Table 1 Effects of myo-inositol and dexamethasone on B(a)P-induced pulmonary adenoma formation in female AJ mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group designation</th>
<th>Preinitiation + initiation periods</th>
<th>Postinitiation period</th>
<th>No. of tumors per mouse</th>
<th>% inhibition</th>
<th>Weight gain (g)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>C-C</td>
<td>None</td>
<td>None</td>
<td>13.5 ± 5.0^a</td>
<td>8</td>
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<tr>
<td></td>
<td>myo-C</td>
<td>myo-inositol</td>
<td>None</td>
<td>10.9 ± 4.3</td>
<td>19</td>
<td></td>
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<tr>
<td></td>
<td>C-myo</td>
<td>None</td>
<td>myo-inositol</td>
<td>9.3 ± 3.8f</td>
<td>31</td>
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<tr>
<td></td>
<td>myo-myo</td>
<td>None</td>
<td>myo-inositol</td>
<td>6.5 ± 3.3h</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C-C</td>
<td>None</td>
<td>None</td>
<td>17.5 ± 6.1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-Dex</td>
<td>None</td>
<td>Dexamethasone</td>
<td>9.3 ± 0.4f</td>
<td>47</td>
<td></td>
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<tr>
<td></td>
<td>Dex-C</td>
<td>None</td>
<td>None</td>
<td>16.5 ± 8.1</td>
<td>7</td>
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<tr>
<td></td>
<td>myo-myo</td>
<td>None</td>
<td>Dexamethasone</td>
<td>11.2 ± 3.9h</td>
<td>32</td>
<td></td>
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<tr>
<td></td>
<td>myo-myo-NNK</td>
<td>None</td>
<td>Dexamethasone</td>
<td>13.2 ± 3.9</td>
<td>27</td>
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</tr>
<tr>
<td>3</td>
<td>C-C</td>
<td>None</td>
<td>None</td>
<td>9.3 ± 0.4f</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>myo-C</td>
<td>None</td>
<td>myo-inositol</td>
<td>5.9 ± 2.6I</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>myo-myo</td>
<td>None</td>
<td>myo-inositol</td>
<td>7.3 ± 2.1I</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(myo + Dex)-(myo + Dex)</td>
<td>myo-inositol + dexamethasone</td>
<td>myo-inositol + dexamethasone</td>
<td>2.3 ± 2.3l</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

Chemicals. The chemicals used were myo-inositol (>99% purity) and dexamethasone (>99% purity; Sigma Chemical Co., St. Louis, MO); B(a)P (>98% purity; Aldrich Chemical Co., Milwaukee, WI); and NNK (>99% purity; National Cancer Institute Carcinogen Repository, Midwest Research Institute, Kansas City, MO).

Animal Experiments. Female AJ mice obtained from the Jackson Laboratory (Bar Harbor, ME) were used in all experiments. The animals were fed a semipurified diet consisting of 27% vitamin-free casein, 59% starch, 10% corn oil, 4% salt mix (USP XIV), and a complete mixture of vitamins (Teklad, Madison, WI). At 7 weeks of age, the mice were randomized by weight into groups of 15. They were weighed at weekly intervals. At 9 weeks of age, the animals were given the initial administration of the carcinogen to be used in the experiment. In experiments in which the carcinogen used was B(a)P, the dose of carcinogen used was 2 mg in 0.2 ml of cottonseed oil given by oral intubation. The administrations of B(a)P were repeated at 4 and 7 days after the initial dose. In animals receiving NNK, the dose was 1.6 mg in 0.1 ml of saline given i.p. Two administrations were given, the second 1 week after the first. Protocols with both carcinogens were terminated 21 weeks after the last dose of carcinogen, at which time the mice were autopsied. Pulmonary adenomas were counted on the surface of the lung using the procedure of Shimkin, as previously described (22, 23). Three administration schedules for the chemopreventive agents were used (Fig. 1). In the first of these, the mice were given the test compounds starting 2 weeks prior to the initial administration of the carcinogen, and the administration of the test compounds was continued until 1 week after the last dose of the carcinogen was given (Fig. 1A). This schedule has been designated "preinitiation and initiation periods." In the second administration schedule, the test compounds were fed starting 1 week after the last administration of carcinogen and were continued for the duration of the protocol (Fig. 1B). This schedule has been designated the "postinitiation period." The third schedule entailed administering the test agents starting 2 weeks prior to the initial administration of the carcinogen and continuing until the end of the experiment (Fig. 1C).

Statistical Analysis. Differences between groups in an experiment were examined by means of ANOVA or, in the case of inhomogeneous variances, by the nonparametric Kruskal-Wallis test. Statistical results by nonparametric comparisons were carried out by means of two-sample t tests with pooled or separate variance estimates, depending on whether the variances were similar (P > 0.2) or different. No adjustment for multiple testing was made for these t tests because each comparison was important on its own. The statistical package SAS was used.

Results

In Table 1, the results of administration of myo-inositol and dexamethasone on B(a)P-induced pulmonary adenoma formation are presented. In experiment 1, the effects of feeding myo-inositol during the three time schedules shown in Fig. 1 are presented. The first schedule (Fig. 1A) entails feeding myo-inositol during the preinitiation and initiation periods [i.e., starting 2 weeks prior to the first administration of B(a)P and continuing until 1 week after the last administration of the carcinogen]. When myo-inositol was fed during this time frame, a small inhibition of pulmonary adenoma formation was found, which was not statistically significant. A larger inhibitory effect occurred when the myo-inositol was fed during the postinitiation period (Fig. 1B). When myo-inositol was given throughout the entire protocol, an additive effect was obtained (Fig. 1C). Experiment 2 demonstrates that dexamethasone fed in the postinitiation period results in a reduction of pulmonary tumors. The data in experiment 3 again show that myo-inositol has a small inhibitory effect when given in the preinitiation and initiation periods. Dexamethasone produces a small but
These diets were continued for the duration of the protocol. Concentrations of the test compound were: myo-inositol, 1%; dexamethasone, 0.5 mg/kg of diet.

**Discussion**

Although cancer of the lungs is the principal cause of cancer deaths in the United States and many other industrialized countries, effective chemoprevention of this neoplasm has not been achieved. In animal models, a number of compounds (blocking agents) can prevent the occurrence of this cancer when administered prior to or simultaneously with exposure to chemical carcinogens, but few are effective when given in the postinitiation period (3, 24). Both of the compounds studied in the present investigation inhibit pulmonary tumor formation when administered in the postinitiation period. When given in the preinitiation and initiation periods, myo-inositol produced a small but statistically insignificant inhibition; dexamethasone had even less effect. The two compounds had not been studied previously under these conditions. Although these data are not definitive, they are of importance in that there is always the possibility that a compound that can inhibit in one stage of the carcinogenic sequence may enhance carcinogenesis when given in another stage. In the studies carried out with myo-inositol and dexamethasone, this certainly is not the case.

The capacity of the glucocorticoid dexamethasone to inhibit pulmonary adenoma formation is of interest in that this compound has minimal systemic effects have been developed and could prove applicable for use as chemopreventive agents in the respiratory tract if administered by aerosol.

Prior studies of inhibition of pulmonary adenoma formation by myo-inositol and dexamethasone have been limited to B(a)P as the carcinogen. In the present work, the two compounds have been shown to inhibit pulmonary carcinogenesis resulting from administrations of NNK in this tumor model. B(a)P and NNK are representative of two major classes of lung carcinogens to which humans are exposed, indicating that myo-inositol and dexamethasone may be useful as chemopreventive agents in this organ site.

**References**

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