SHORT COMMUNICATION

Studies of chemopreventive effects of budenoside on benzo[a]pyrene-induced neoplasia of the lung of female A/J mice

Lee W. Wattenberg1 and Richard D. Estensen

Department of Laboratory Medicine and Pathology, 6-133 Jackson Hall, University of Minnesota, Minneapolis, MN 55455, USA

1To whom correspondence should be addressed

The objective of the present investigation was to determine conditions under which the synthetic glucocorticoid, budenoside, will inhibit benzo[a]pyrene (BaP)-induced pulmonary carcinogenesis when administered in the post-initiation period. For this purpose, female A/J mice were employed. The animals were given three administrations of 2 mg of BaP by oral intubation during a 1-week period. Budenoside was fed in the diet subsequent to the last dose of BaP. Using this format, two experiments were carried out to determine the effects of varying the time of administration of budenoside on the magnitude of the inhibition obtained. In both experiments, one group of mice was fed budenoside (1.5 mg/kg diet) from 1 week after the last dose of BaP until the termination of the experiment, 15 weeks later. The reduction of pulmonary tumor formation under these conditions was 89% in the first experiment and 78% in the second (average 84%). In the first experiment the effects of feeding budenoside only during weeks 1–5 after BaP administration was studied. Under these conditions, inhibition of pulmonary tumor formation was 35%. In the second experiment, the effects of postponing the start of feeding budenoside was determined. In mice in which the budenoside feeding was delayed until 5 weeks after the last dose of BaP and then continued for the duration of the protocol, a 67% inhibition of tumor formation was found. The data obtained indicate that budenoside will produce inhibition of pulmonary adenoma formation when fed either early or late in the post-initiation stage of carcinogenesis, and that feeding throughout the entire post-initiation period gives maximum inhibition.

Introduction

This investigation is part of a continuing effort to develop effective chemoprevention of carcinogenesis of the lungs. A particular focus has been on compounds that will inhibit when administered in the post-initiation period. Few compounds have been found to have this property for pulmonary carcinogenesis (1–6). One such is the synthetic glucocorticoid, dexamethasone. This compound has been shown to inhibit benzo[a]pyrene (BaP)- and 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced pulmonary adenoma formation in female A/J mice when administered subsequent to the last dose of carcinogen (6,7). In addition to the lung, dexamethasone also has been shown to inhibit carcinogenesis of the forestomach and skin of the mouse when given in the post-initiation period (6,8,9). Dexamethasone has a large number of biological effects, which can account for its inhibitory properties (10–14). In the case of pulmonary adenoma formation, its capacity to mature type 2 alveolar cells, the major cell type occurring in pulmonary adenomas in the experimental model used in the present study, is particularly noteworthy. Which effect or combination of effects is responsible for the cancer prevention properties of dexamethasone in the lungs as well as other tissues has not been established. The study to be presented employs a second synthetic glucocorticoid, budenoside (15–17). This investigation demonstrates that this compound prevents pulmonary adenoma formation when administered throughout the post-initiation period. In addition, its capacity to inhibit when administered during only a portion of the post-initiation period also has been evaluated.

Materials and methods

Animal experiments

Two experiments were performed. In both, female A/J mice obtained from the Jackson Laboratories (Bar Harbor, ME) were used. The animals were fed a semi-purified diet containing 27% vitamin free casein, 59% starch, 10% corn oil, 4% salt mix (USP XIV) and a complete mixture of vitamins (Teklad, Madison, WI). At 9 weeks of age, the animals were given an initial administration of the carcinogen BaP by oral intubation. Administrations of BaP were repeated at 4 and 7 days after the initial procedure. The dose of BaP was 2 mg per 20 g body wt in 0.2 ml of cottonseed oil. The mice were randomized by weight following the last administration of BaP and were reweighed at weekly intervals. Budenoside was fed in the diet in the post-initiation period as described for the individual experiments. In the first experiment, there were three groups of mice. One group was fed budenoside from 1 to 5 weeks after the last dose of BaP. The second was fed the compound from 1 to 15 weeks after BaP, and the third was not given budenoside. In the second experiment, the feeding of budenoside was begun at various intervals subsequent to the last administration of BaP and then was continued until the end of the protocol which was 16 weeks after the last dose of BaP. The time of starting the budenoside feeding varied from 1 day to 5 weeks after the last dose of BaP. At the termination of both experiments, the animals were killed and autopsied. The lungs were taken for pulmonary tumor counts. The pulmonary adenomas were counted on the surface of the lung using the procedure of Shimkin as previously described (18,19).

Chemicals

The chemicals used were BaP (>98% purity) (Aldrich Chemical Co., Milwaukee, WI) and budenoside (>98% purity) (Sigma Chemical Co., St. Louis, MO).

Statistical analyses

Differences between groups in an experiment were examined by the non-parametric Kruskal–Wallis test due to inhomogeneous differences. If the overall test was significant, pairwise comparisons were carried out. The statistical package SAS was used.

Results

Both experiments showed that feeding budenoside from 1 to 15 weeks after the last dose of BaP inhibited pulmonary adenoma formation. In addition, they provided data as to the effects of the timing of budenoside administration on pulmonary adenoma formation. In experiment 1, the effects of restricting the feeding of budenoside to an early interval in the post-initiation period, i.e. from 1 to 5 weeks after the last dose of carcinogen, was determined. Under these conditions,
Effects of budenoside on BaP-induced pulmonary adenoma formation in female A/J mice

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Dietary additions</th>
<th>Period of diet feedinga</th>
<th>Duration of post-initiation period (weeks)</th>
<th>Pulmonary adenomas</th>
<th>Weight gain (g)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start</td>
<td>End</td>
<td>Tumors per % inhibition</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>1</td>
<td>5</td>
<td>16</td>
<td>11.9 ± 3.7a</td>
</tr>
<tr>
<td>1</td>
<td>Budesonide</td>
<td>1</td>
<td>16</td>
<td></td>
<td>7.7 ± 2.8d</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>–</td>
<td>–</td>
<td></td>
<td>1.3 ± 1.4e</td>
</tr>
<tr>
<td>2</td>
<td>Budesonide</td>
<td>–</td>
<td>–</td>
<td></td>
<td>17.4 ± 5.2</td>
</tr>
<tr>
<td>15</td>
<td>Budesonide (1 day)</td>
<td>16</td>
<td></td>
<td></td>
<td>1.7 ± 1.6f</td>
</tr>
<tr>
<td>15</td>
<td>Budesonide</td>
<td>1</td>
<td>16</td>
<td></td>
<td>3.8 ± 2.1g</td>
</tr>
<tr>
<td>15</td>
<td>Budesonide</td>
<td>2</td>
<td>16</td>
<td></td>
<td>5.7 ± 2.9h</td>
</tr>
<tr>
<td>15</td>
<td>Budesonide</td>
<td>5</td>
<td>16</td>
<td></td>
<td>5.7 ± 2.7i</td>
</tr>
</tbody>
</table>

aAt 9 weeks of age, female A/J mice were given an initial dose of 2 mg BaP per 20 g body wt in 0.2 ml cottonseed oil by oral intubation. The administrations were repeated 4 and 7 days after the initial procedure. Following the last administration of BaP, mice were fed diets containing budenoside 1.5 mg/kg of diet during the interval shown in the columns headed ‘Period of diet feeding’.

bWeight gain from time of randomization of the mice, which was 1 day after the last dose of BaP until completion of the experiment.

Table I.

Discussion

Although cancer of the lung is the principal cause of cancer deaths in the United States and many other industrialized countries, effective chemoprevention for 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 post-initiation period (1–7). Two glucocorticoids, budenoside and dexamethasone, have now been shown to have the capacity to inhibit pulmonary tumor formation when administered in the post-initiation period. A problem with the use of glucocorticoids as chemopreventive agents is the occurrence of systemic effects. Further work is required to determine if efficacy can be obtained under conditions that minimize this complication.

Acknowledgement

The financial assistance of the Gerald T. Evans Fund is gratefully acknowledged.

References


*Received on March 17, 1997; revised on May 22, 1997; accepted on June 12, 1997*