Inhibiting Effect of Caffeine on Spontaneous and Urethan-induced Lung Tumors in Strain A Mice

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ABSTRACT

The i.p. injection of caffeine (8, 20, and 40 mg/kg) 3 times weekly for 8 weeks suppressed the development of spontaneous pulmonary adenomas in strain A mice. The same caffeine injection scheme suppressed urethan (0.25 and 1.0 mg/g)-induced lung tumor development when caffeine treatment started 1 week before urethan administration, but this suppression was not significant when caffeine treatment was initiated 1 week after urethan injection. The most pronounced suppression of lung tumor formation occurred when caffeine was given as only two injections 3 hr before and 3 hr after urethan administration. The incorporation of [3H]thymidine into lung tissue DNA of caffeine-treated mice was impaired at the time of urethan administration. Also, caffeine partially antagonized the effects of urethan on lung tissue, as measured by [3H]thymidine incorporation studies. One interpretation of these results is that caffeine-induced suppression of DNA synthesis interferes with pulmonary adenoma induction by decreasing the affinity of lung tissue DNA for urethan. The finding that chronic caffeine treatment produced continued suppression of [3H]thymidine incorporation into lung tissue DNA suggests that caffeine-induced inhibition of spontaneous pulmonary adenoma formation is due to a general suppression of lung DNA-synthetic activity.

INTRODUCTION

Caffeine has been reported by several authors to antagonize the carcinogenic effect of chemicals in vivo and in vitro. Caffeine suppressed the carcinogetic effect of cigarette smoke condensate on mouse skin (12) and antagonized 4-nitroquinoline 1-oxide (9) and antagoized lung tumorogenesis in mice. In vitro caffeine inhibited 4-nitroquinoline 1-oxide-induced transformation of BALB/3T3 mouse cells (5).

It has been proposed that the anticarcinogenic effect of caffeine is due to a potentiation of the lethal effects of chemical carcinogens, leading to the death of cells that would have produced a tumor if they had survived (5, 9, 10). This proposal is based on the demonstration of a caffeine-sensitive postreplication repair process in cells in which DNA is damaged by various chemicals (2, 4, 11, 16, 18). Nontoxic concentrations of caffeine suppress postreplication repair in cells exposed to these chemicals, resulting in enhanced chromosomal damage that leads to increased cell lethality.

MATERIALS AND METHODS

Effect of Caffeine on Spontaneous Pulmonary Carcinogenesis. Six-week-old male strain A mice (L. C. Strong Research Foundation, San Diego, Calif.) were used as test animals. From a preliminary toxicity test performed as described previously (15), a MTD was determined for caffeine (Calbiochem, San Diego Calif.). The MTD is defined as the dose that all 5 treated mice tolerated after receiving 9 i.p. injections of caffeine dissolved in 0.85% NaCl solution over a 3-week period (mice were held for 4 weeks to detect any delayed toxicity).

Groups of 40 mice were given injections i.p. 3 times weekly for a total of 24 injections with the MTD, one-half the MTD, and one-fifth the MTD of caffeine; one group of mice were given injections of only the 0.85% NaCl solution vehicle (Table 1). Twenty-four weeks after the first injection, the mice were sacrificed, and the lungs were placed in Tellyesniczky’s fluid. After 48 hr, the lungs were examined under a Spencer dissecting microscope (Spencer Lens Co., Buffalo, N. Y.) (×10), and the number of surface adenomas was counted. A few surface nodules were examined histologically to confirm the typical morphological appearance of the adenoma. The frequency of lung tumors in each caffeine-treated group was statistically compared with that in the vehicle-treated control group by the standard Student’s t test.

Effect of Caffeine on the Pulmonary Tumor Response to Urethan. In this experiment (Table 2), groups of 20 mice were subjected to single s.c. injections of urethan (0.25 or 1.0 mg/g; Matheson, Coleman & Bell, Norwood, Ohio) dissolved in 0.85% NaCl solution, as well as being given i.p. injects of caffeine (40 or 20 mg/kg). In 1 series, caffeine treatment was initiated 7 days after urethan injection; caffeine was injected 3 times weekly for a period of 8 weeks. In a second series, caffeine treatment was initiated 7 days before urethan injection; caffeine was injected 3 times weekly for 8 weeks. In a third series, caffeine treatment occurred only on the same day as urethan injection; caffeine was injected 3 hr before and 3 hr after urethan. Sixteen weeks after the urethan injection, the mice were sacrificed, and the number of adenomas appearing on the

1 This research was supported by Contract NIH-N01-CP-33232 from the National Cancer Institute.

Received January 5, 1978; accepted March 14, 1978.

To investigate further the anticarcinogenic effect of caffeine, we have assessed the effects of this agent on the development of spontaneous and urethan-induced lung tumors in strain A mice. These studies have been coupled with an investigation of the effects of caffeine on [3H]dThd incorporation into lung tissue DNA.

The abbreviations used are: dThd, thymidine; MTD, maximum tolerated dose.

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surface of the lungs was quantitated as described previously. The frequency of lung tumors in each caffeine-treated group was statistically compared to the corresponding group that received urethan alone.

**Effect of Caffeine on the Incorporation of [3H]dThd into Lung Tissue DNA.** In these 2 experiments (Chart 1), 1 group of mice was given i.p. injections of caffeine (40 mg/kg) 3 hr before and 3 hr after sham urethan administration (0.85% NaCl solution), and a second group of mice was given i.p. injections of caffeine (40 mg/kg) every second day, starting 7 days before sham urethan administration. A group of mice was also treated with caffeine alone, and another group of mice was treated with urethan alone. Initiation of caffeine treatment 7 days prior to urethan exposure resulted in a significant suppression of the pulmonary tumor response to either the low or the high dose of urethan. Initiation of caffeine treatment 7 days prior to urethan exposure resulted in a significant suppression of the pulmonary tumor response to the high dose of urethan. When the caffeine treatment consisted of only 2 injections given 3 hr before and 3 hr after urethan, the lung tumor response was significantly suppressed at both the high and the low doses of urethan.

**Effect of Caffeine on the Pulmonary Tumor Response to Urethan.** The effect of caffeine on the incidence of urethan-induced pulmonary adenomas is depicted in Table 2. All mice treated with caffeine again survived for the duration of the experiment, and none of these mice lost weight as a result of caffeine treatment. When caffeine treatment was initiated 7 days after exposure to urethan, there was no significant suppression of the pulmonary tumor response to either the low or the high dose of urethan. Initiation of caffeine treatment 7 days prior to urethan exposure resulted in a significant suppression of the lung tumor response to the high dose of urethan. When the caffeine treatment consisted of only 2 injections given 3 hr before and 3 hr after urethan, the lung tumor response was significantly suppressed at both the high and the low doses of urethan.

**Table 1.**

<table>
<thead>
<tr>
<th>Caffeine dose (mg/kg)</th>
<th>No. of adenomas/mouse</th>
<th>% control adenomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.23 ± 0.06</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>0.08 ± 0.08</td>
<td>35</td>
</tr>
<tr>
<td>20</td>
<td>0.06 ± 0.04</td>
<td>26</td>
</tr>
<tr>
<td>40</td>
<td>0.05 ± 0.04</td>
<td>22</td>
</tr>
</tbody>
</table>

* Effects of caffeine on spontaneous pulmonary carcinogenesis in strain A mice.

* Caffeine dissolved in 0.85% NaCl solution injected i.p. 3 times weekly for 8 weeks.

* Mean ± S.E.

* p < 0.05.

**Table 2.**

<table>
<thead>
<tr>
<th>Urethan dose (mg/g)</th>
<th>Caffeine dose (mg/kg)</th>
<th>Day +7*</th>
<th>No. of adenomas/mouse</th>
<th>% control</th>
<th>Day -7*</th>
<th>No. of adenomas/mouse</th>
<th>% control</th>
<th>Day 0*</th>
<th>No. of adenomas/mouse</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0</td>
<td>2.5 ± 0.4</td>
<td>100</td>
<td>2.5 ± 0.4</td>
<td>100</td>
<td>2.5 ± 0.4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>2.4 ± 0.4</td>
<td>96</td>
<td>2.7 ± 0.4</td>
<td>100</td>
<td>2.5 ± 0.4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>2.0 ± 0.4</td>
<td>80</td>
<td>1.9 ± 0.3</td>
<td>76</td>
<td>1.2 ± 0.2</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>13.7 ± 1.3</td>
<td>100</td>
<td>13.7 ± 1.3</td>
<td>100</td>
<td>13.7 ± 1.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>14.4 ± 1.3</td>
<td>105</td>
<td>8.5 ± 1.0</td>
<td>62</td>
<td>10.0 ± 0.9</td>
<td>73</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0.25</td>
<td>10.5 ± 0.2</td>
<td>77</td>
<td>8.1 ± 0.7</td>
<td>59</td>
<td>9.2 ± 1.1</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Caffeine treatment initiated 7 days after s.c. urethan injection; caffeine was injected i.p. 3 times weekly for a period of 8 weeks.

* Caffeine treatment initiated 7 days before s.c. urethan injection; caffeine was injected i.p. 3 times weekly for a period of 8 weeks.

* Caffeine treatment only on the same day as s.c. urethan injection; caffeine was injected i.p. 3 hr before and 3 hr after urethan.

* Mean ± S.E.

* p < 0.01.

* p < 0.02.

* p < 0.05.
The low dose of urethan produced a maximum suppression of lung DNA-synthetic activity of the lungs gradually decreased with a maximum of approximately 35% at the time when urethan would have been administered. When caffeine was given only as 2 injections spaced 6 hr apart, the suppression of lung [3H]-dThd-incorporating activity reached a maximum of 63% by Day 1. This was followed by a recovery of lung [3H]-dThd-incorporating activity, which was normal by Day 5 and which peaked at 158% of normal [3H]-dThd-incorporating activity on Day 8. When caffeine treatment was initiated 7 days before sham urethan administration and continued throughout the course of the experiment, the [3H]-dThd-incorporating activity of the lungs gradually decreased with a maximum suppression of 71% by Day 9.

**Effect of Caffeine on the [3H]-dThd Incorporation Into Lung DNA of Urethan-treated Mice.** The effect of caffeine on urethan-induced alterations in lung tissue DNA synthetic activity is depicted in Chart 2. For demonstration of the effect of a high and a low dose of urethan on lung tissue DNA-synthetic activity, the [3H]-dThd incorporation is represented in this chart as a percentage of the untreated control [3H]-dThd incorporation. For demonstration of the effect of the MTD of caffeine on urethan-induced alterations in lung DNA-synthetic activity, the [3H]-dThd incorporation into the lung DNA of mice treated with both urethan and caffeine is represented as a percentage of the [3H]-dThd incorporation occurring in mice treated with caffeine alone. By normalizing the data in this fashion, it is possible to compare urethan-induced alterations in the lung DNA-synthetic activity of otherwise untreated mice with urethan-induced alterations in the lung DNA-synthetic activity of caffeine-treated mice.

Both the high and the low doses of urethan suppressed lung tissue [3H]-dThd-incorporating activity over the first 24 hr after urethan administration. Lung [3H]-dThd-incorporating activity returned to normal within 5 days at both urethan doses and subsequently increased above the normal lung [3H]-dThd incorporation level, with a peak in activity at 8 days. The low dose of urethan produced a maximum suppression in [3H]-dThd-incorporating activity to 61% of normal and a maximum enhancement to 174% of normal [3H]-dThd-incorporating activity. The high dose of urethan induced greater alterations in lung [3H]-dThd-incorporating activity, producing a maximum suppression to 33% of normal and a maximum enhancement to 385% of normal DNA-synthetic activity.

Caffeine antagonized the effect of urethan on lung tissue DNA synthesis as measured by [3H]-dThd incorporation. Both the early suppression and the later enhancement of [3H]-dThd-incorporating activity produced by the low dose of urethan were prevented by caffeine treatment. In the group of mice treated with the high dose of both urethan and caffeine, the maximum suppression of [3H]-dThd incorporation was only to 62% of the level in mice treated with caffeine alone, and the maximum enhancement was only to 255% of the level in mice treated only with caffeine.

**DISCUSSION**

The results of these studies demonstrate that caffeine can suppress both spontaneous and urethan-induced pulmonary adenoma development in mice (Tables 1 and 2). The finding that initiation of caffeine treatment 7 days after urethan administration did not significantly suppress lung tumor development, coupled with the fact that the most pronounced suppression of lung tumor formation occurred when caffeine was given as only 2 injections 3 hr before and 3 hr after urethan administration (Table 2), suggests that the anticarcinogenic effect of caffeine is due to interference with the ability of urethan to induce pulmonary adenomas rather than to suppression of the growth of these urethan-induced tumors.
The $[^3H]dThd$ incorporation studies (Chart 1) illustrate that caffeine given either by a chronic injection regimen (3 times weekly) or by an acute injection regimen (2 injections spaced 6 hr apart) resulted in depression of the DNA-synthetic activity of the lungs at the point in time when urethan administration occurred. This depression of DNA-synthetic activity was transient in mice that were given the acute caffeine injection regimen and prolonged in mice that were given the chronic caffeine injection regimen.

The finding that urethan produced an early suppression and a later enhancement of the DNA-synthetic activity of lung tissue (Chart 2) agrees with previously published results (6, 14). Shimkin et al. (14) found that, while a high dose of urethan produced this effect, urethan at a low but still carcinogenic dose did not. They also found that a carcinogenic dose of isopropyl carbamate did not significantly inhibit DNA synthesis early after treatment but did produce a later enhancement. Carcinogenic doses of nitrogen mustard and methylcholanthrene produced an early inhibition of DNA synthesis but no significant rebound later on. These results led Shimkin et al. to suggest that the early suppression and later enhancement of lung tissue DNA-synthetic activity is not a key event in the carcinogenic process but may represent a toxic side effect of urethan. This conclusion was supported by Kauffman (6), who found that type 2 alveolar epithelial cells, the cell type from which pulmonary adenomas develop, comprised such a small fraction (12%) of the total lung cell population labeled with $[^3H]dThd$ in labeling index studies that neither the early suppression nor the later enhancement of DNA-synthetic activity induced by urethan could be due to alterations in the type 2 cell population. Kauffman concluded that the urethan-induced early inhibition and later enhancement of DNA-synthetic activity is due to alterations in the lung population of nonvacuolated alveolar cells and that these alterations seem to reflect a normal response to tissue injury rather than a pathological one.

We have demonstrated that caffeine antagonizes the effects of urethan on lung DNA synthesis, as measured by $[^3H]dThd$ incorporation studies (Chart 2). In light of the work of Shimkin et al. (14) and Kauffman (6) described previously, one interpretation of these results is that caffeine partially prevents urethan-induced injury to the lung.

One of the possible ways in which caffeine may impair urethan-induced lung injury is by competition of caffeine with lung tissue DNA for the binding of urethan. This explanation is based on the finding that caffeine readily binds with carcinogens such as 3,4-benzopyrene (8), dibenzcarbazoles and dibenzacridines (1), and polycyclic aromatic hydrocarbons (17). By binding to urethan, caffeine would decrease the amount of urethan that is free to react with lung tissue DNA.

Another possible explanation for the caffeine-induced antagonism of urethan toxic effects on the lung is that caffeine decreases the binding time of urethan to lung DNA by decreasing the DNA-synthetic activity of lung tissue. The length of the binding time of ethyl carbamate (urethan) to DNA has been related to the DNA-synthetic activity of the target organ by the demonstration that the binding of this agent to mouse liver DNA persisted longer in partially hepatectomized than in intact mice (7). It is thus possible that the decreased lung DNA-synthetic activity of caffeine-treated mice at the time of urethan administration (Chart 1) leads to a decrease in the binding time of urethan to lung DNA.

Whether the antagonism between urethan and caffeine is due to the binding of caffeine to urethan or to the suppression of DNA synthesis by caffeine, the finding that caffeine partially prevents urethan-induced injury to the lung does not fit in with the postreplication repair model for the anticarcinogenic effects of caffeine described in the introduction to this paper. According to this model, caffeine should enhance the toxic effects of urethan on lung tissue. A more probable explanation for the results described in this paper is that, under the conditions in which these experiments were performed, caffeine suppresses urethan-induced pulmonary adenoma formation by interfering with the interaction between urethan and lung tissue. This explanation does not preclude the validity of the postreplication repair model. It does suggest, however, that caffeine may produce anticarcinogenic effects by more than 1 mechanism.

It would be difficult to derive a conclusion concerning the mechanism by which caffeine suppresses spontaneous pulmonary adenoma formation based on the data presented in this paper. However, the finding that the caffeine injection scheme that suppressed spontaneous lung tumor development (Table 1) also produced a continued suppression of $[^3H]dThd$ incorporation into lung DNA (Chart 1) does suggest that this suppression of lung DNA-synthetic activity may play a role in caffeine-induced inhibition of spontaneous pulmonary adenoma formation.

ACKNOWLEDGMENTS

The authors are indebted to Donald D. Hopfenstock, Linda S. Terry, Mamie C. Troxell, and Stephen H. Parker for their excellent technical assistance.

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