Recovery From 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone-Induced Immunosuppression in A/J Mice by Treatment With Nonsteroidal Anti-inflammatory Drugs

Nathalie Rioux, Andre Castonguay*

Background: We have previously reported that nonsteroidal anti-inflammatory drugs inhibit lung tumorigenesis induced by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in mice. Purpose: The aims of this study were to determine if NNK suppresses humoral (i.e., antibody) and cellular immune responses in mice and if nonsteroidal anti-inflammatory drugs could attenuate these immune responses. Methods: Female A/J mice (7-8 weeks old) were fed nonsteroidal anti-inflammatory drugs starting 2 weeks before the beginning of NNK treatment (9.1 mg per mouse in total) and continuing through the 7 weeks of NNK treatment. Eight groups (two control groups and six experimental groups) of 10 mice each were used per experiment. Animals in the two control groups received the same diet and water as animals in the six experimental groups; one control group received no nonsteroidal anti-inflammatory drugs or NNK and the other control group received only NNK. The primary humoral and cellular immune responses to the various treatments were assayed by the plaque-forming cell technique and by measurement of natural killer cell cytotoxic activity, respectively. At the end of each experiment, the animals were killed, blood was collected, plasma was prepared, and levels of the immune system modulator prostaglandin E₂ were measured. Results: NNK treatment inhibited the plaque-forming cell response by approximately 50%; this inhibition was attenuated by treatment with sulindac or acetylsalicylic acid (P = .0001 for both). In contrast, treatment with naproxen, which had no chemopreventive (i.e., tumor inhibitory) efficacy, further increased by 26% (P = .05) the immunosuppressive effect of NNK. The cytotoxic activity of splenic natural killer cells against YAC-1 cells was reduced by 60% (P = .002); treatment with acetylsalicylic acid (254 mg/kg of diet) reduced the NNK-induced natural killer cell cytotoxicity inhibition by 50% (P = .02), whereas the administration of the specific cyclooxygenase-2 inhibitor NS-398 (7 mg/kg of diet) resulted in an almost complete recovery (approximately 95%, P = .04) of natural killer cell activity. The prostaglandin E₂ plasma concentration was approximately 100% greater in NNK-treated mice than in untreated mice. Treatment of the mice with nonsteroidal anti-inflammatory drugs attenuated this elevation (from approximately 25% to 100%), and NS-398 (7 mg/kg of diet) was the most effective (100%). Conclusions and Implications: The ability of various nonsteroidal anti-inflammatory drugs to inhibit NNK-induced carcinogenesis appears to be directly related to the ability of these drugs to inhibit NNK-induced immunosuppression. Our results suggest that the chemopreventive effect of nonsteroidal anti-inflammatory drugs may be mediated through the modulation of prostaglandin E₂ synthesis. [J Natl Cancer Inst 1997;89:874-80]

Following the lack of progress in lung cancer therapy (1), chemoprevention (i.e., administration to individuals at risk of cancer of nontoxic agents that inhibit carcinogenesis) has been suggested as a promising approach to cancer control (2). One of the most interesting classes of chemopreventive agents are the nonsteroidal anti-inflammatory drugs. In 1991, about 70.3 million prescriptions for nonsteroidal anti-inflammatory drugs were filled across the United States to treat arthritis and related musculoskeletal problems (3). These agents are usually taken daily for periods up to several years (4). Gastrointestinal toxic effects are observed following long-term treatments with conventional nonsteroidal anti-inflammatory drugs (5). NS-398, a new selective inhibitor of cyclooxygenase-2, has the advantage of being far less ulcerogenic (6). Epidemiologic studies (7-9) concluded that long-term use of aspirin, the prototype of nonsteroidal anti-inflammatory drugs, reduced the risk in humans of gastrointestinal cancer, especially colon cancer (7-9). Thun et al. (7) did not observe a statistically significant inhibition of lung cancer following aspirin use. Another nonsteroidal anti-inflammatory drug, sulindac, has been recommended in the treatment of large-bowel polyps in patients with familial polyposis (10-12). Sulindac and aspirin also have shown antiproliferative effects against human colon cancer cells in vitro (13).

Numerous laboratory studies (14-26) preceded these epidemiologic surveys, and the results suggested prophylactic properties for nonsteroidal anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs, such as aspirin, indomethacin, and sulindac, have been found to inhibit chemically induced tumorigenesis of various organs (14-23) in laboratory animals. Castonguay and co-workers (24-26) were the first to compare the efficacy of nonsteroidal anti-inflammatory drugs against lung carcinogenesis in laboratory animals; of the agents tested, sulindac was the most effective and naproxen had no preventive effects (25). A hypothesis involving inhibition of arachidonic acid metabolism by the cyclooxygenase pathway has been proposed (14).

Rodent and human colon tumors contain high levels of prostaglandins, especially prostaglandin E₂ (PGE₂) (27). PGE₂ is a potent modulator of functions of most of the cells of the immune system (28). Cyclooxygenase inhibitors such as nonsteroidal anti-inflammatory drugs abolish PGE₂ synthesis and attenuate immune suppression: Aspirin enhances T-cell proliferation and interleukin-2 production, whereas indomethacin augments lymphokine-activated killer cell generation in cancer patients (29-31). Nevertheless, the mechanism(s) of cancer chemoprevention by nonsteroidal anti-
inflammatory drugs remain(s) unknown.

Inhibition of the immune response by tobacco smoking may predispose smokers to infections and cancer. In smokers, natural killer cell activity is depressed, and helper and suppressor T-cell ratios and mitogen-induced lymphocyte transformation are reduced. Alveolar macrophage effector functions are also partially suppressed in smokers (32). Serum levels of immunoglobulin (Ig) G, IgM, and IgA in smokers are reduced by 10%-20% (33,34). Briefly, tobacco smoking affects both specific and nonspecific humoral immunity and cellular immunity. The mechanism by which tobacco smoking suppresses the immune response has not been established.

Tobacco smoke contains at least 43 compounds known to induce tumors in laboratory animals, and tobacco smoking is a well-established risk factor for lung cancer (reviewed in (35)). One of the most potent of the carcinogens found in tobacco smoke is nicotine-derived 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which has a remarkable specificity for the lungs in laboratory animals, and tobacco smoking and thereby could provide further insight into the understanding of the mechanism of cancer chemoprevention by these drugs.

**Materials and Methods**

**Chemicals.** NNK with a purity of 99% (evaluated by thin-layer chromatography) was purchased from Chemsys Science Laboratory, Lenexa, KS. The nonsteroidal anti-inflammatory drugs (hereafter termed NSAIDs), sulindac, acetylsalicylic acid, and naproxen were purchased from Sigma Chemical Co., St. Louis, MO. NS-398 was obtained from Biomol Research Laboratories, Plymouth, PA. RPMI-1640 medium, fetal bovine serum, and penicillin-streptomycin solution were purchased from Life Technologies, Inc. (GIBCO BRL), Burlington, ON, Canada. Hanks’ balanced salt solution was purchased from Sigma Chemical Co. Guinea pig complement was purchased from Cedarslane Laboratories, Hornby, ON. Ficoll–Paque was obtained from Pharmacia Biotech, Montreal, PQ, Canada. The lactate dehydrogenase release enzyme immunoassay kit was purchased from Boehringer Mannheim, Montreal. The [5,6,8,11,12,14,15-3H(N)]PGE2 was supplied by Du Pont NEN, Mississauga, ON.

**Animals.** Female A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. Animal experiments were done in accordance with approved institutional protocols and by use of the guidelines of the Canadian Council of Animal Care. The mice were maintained under specific-pathogen-free conditions (five mice per cage; 22 °C ± 1 °C; 28% ± 5% relative humidity; 12-hour light–dark cycle). Mice were sorted on the basis of weight, and treatments were randomized by cage. In the first experiment, mice were fed 2 weeks with or without NSAIDs and used for a plaque-forming cell assay. For the other assays (plaque-forming cells, natural killer cell cytotoxicity, and plasma PGE2), groups of 10 mice each were administered NNK at a cumulative dose of 9.1 mg in tap water per mouse for a period of 7 weeks. The control group of mice was given tap water only. Animals were fed a powdered AIN-76A diet with or without (control animals) NSAIDs for 2 weeks before (week −2 to week 0) and during the carcinogen treatment (week 0 to week +7). NNK was given at an initial concentration of 62.4 μg/mL. Consumption of NNK was monitored and adjusted twice weekly for 7 weeks. Animals were killed 1 week after the end of NNK treatment (week +8). Details of the treatment with NNK and NSAIDs are included in Tables 1 and 2. One week after NNK treatment, mice were anesthetized with a solution of ketamine (2 mg/mouse) and xylazine (0.25 mg/mouse) and killed by cervical dislocation.

**Diet.** The AIN-76A powdered diet was purchased from Teklad Premium, Madison, WI. Powdered NSAIDs were mixed with the diet in a V-blender for 1 hour to obtain a homogeneous preparation. Diets containing acetylsalicylic acid were prepared weekly, and diets containing other NSAIDs were prepared every 2 weeks. Diets were stored in the dark in sealed containers at 4 °C. The doses of NSAIDs used were equivalent to therapeutic doses used for the treatment of rheumatoid arthritis and osteoarthritis in humans (39). Animals had access to food and water ad libitum, and the powder feeders (Laboratory Product Corp., Maywood, NJ) were replenished with fresh diet twice a week. Diet consumption was monitored three times (weeks −1, +2, and +5) during carcinogen treatment.

**Cell culture.** The YAC-1 murine lymphoma, induced by inoculation of the Moloney leukemia virus into a newborn A/J mouse, was a gift from the Institut Armand-Frappier, Montreal (40). This cell line was grown on plastic dishes in RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 IU/mL penicillin, and 10 IU/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2.

**Direct hemolytic plaque-forming cell technique.** Three days after the end of 7 weeks of NNK treatment, groups of 10 mice each were given an intraperitoneal injection of 5 × 109 fresh sheep red blood cells in saline solution. Four days later, spleens were removed and teased through a 70-μm nylon mesh into Hanks’ balanced salt solution. A 100-μL aliquot of a 1:40 dilution of the splenocyte suspensions and 100 μL of sheep red blood cells (3 × 107/mL) were added to 1 mL of a 0.7% agarose solution at 45 °C. The cells were plated and incubated for 90 minutes at 37 °C. One milliliter of a 1:10 dilution of guinea pig complement was added, and the incubation was continued for another 90 minutes.

Table 1. Effects of 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and nonsteroidal anti-inflammatory drugs (NSAIDs) on food consumption and body weight gain of A/J mice

<table>
<thead>
<tr>
<th>NSAIDs</th>
<th>NNK, mg/mouse†</th>
<th>NSAIDs, mg/kg of diet (mmol/kg of body weight)</th>
<th>Food consumption, g/mouse per day‡</th>
<th>Body weight gain, g/mouse§</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>2.63 ± 0.03</td>
<td>+21.9 ± 0.2</td>
</tr>
<tr>
<td>None</td>
<td>9.09 ± 0.01</td>
<td>None</td>
<td>2.94 ± 0.13</td>
<td>+6.1 ± 5.6†</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>9.11 ± 0.01</td>
<td>254 (199)</td>
<td>2.83 ± 0.18</td>
<td>−2.5 ± 5.9</td>
</tr>
<tr>
<td>Naproxen</td>
<td>9.07 ± 0.12</td>
<td>230 (164)</td>
<td>3.29 ± 0.34</td>
<td>−3.8 ± 5.6</td>
</tr>
<tr>
<td>Sulindac</td>
<td>9.08 ± 0.02</td>
<td>123 (50)</td>
<td>2.90 ± 0.51</td>
<td>−5.8 ± 5.7§</td>
</tr>
<tr>
<td>Sulindac</td>
<td>9.10 ± 0.02</td>
<td>61 (23)</td>
<td>2.74 ± 0.28</td>
<td>−3.4 ± 2.7§</td>
</tr>
<tr>
<td>Sulindac</td>
<td>9.09 ± 0.02</td>
<td>30 (18)</td>
<td>3.26 ± 0.36</td>
<td>−2.4 ± 5.4§</td>
</tr>
<tr>
<td>NS-398</td>
<td>9.09 ± 0.02</td>
<td>7 (3)</td>
<td>3.01 ± 0.32</td>
<td>−2.2 ± 3.0§</td>
</tr>
</tbody>
</table>

*Seven-week-old mice were given a total dose of 9.1 mg of NNK in drinking water for 7 weeks (weeks 0 to +7) and NSAIDs for 9 weeks (weeks −2 to +7). †Mean ± standard deviation (n = 10); standard deviation was less than 1.5% of the mean. ‡Mean ± standard deviation from three values (weeks −2, +3, and +7). §+ = gain; − = loss. From week −2 to week +7, mean ± standard deviation (n = 4).

Statistically different from untreated group, Student’s t test, two-sided P < 0.01.

**Table 1. Effects of 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and nonsteroidal anti-inflammatory drugs (NSAIDs) on food consumption and body weight gain of A/J mice**
Table 2. Effects of 2 weeks of exposure to nonsteroidal anti-inflammatory drugs (NSAIDs) or 7 weeks of exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) with or without feeding NSAIDs on responses of plaque-forming cells (PFC) to sheep red blood cells*

<table>
<thead>
<tr>
<th>NSAIDs</th>
<th>NSAIDs, mg/kg of diet</th>
<th>NNK, mg/mouse</th>
<th>PFC per million cells, mean ± SE†</th>
<th>PFC per spleen, mean ± SE†</th>
<th>PFC per million cells, P‡</th>
<th>PFC per spleen, P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>None</td>
<td>195 ± 8</td>
<td>15 780 ± 1630</td>
<td>nd</td>
<td>92 ± 11</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>9.1</td>
<td>nd</td>
<td>nd</td>
<td>135 ± 8§</td>
<td>68 ± 6§</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>254</td>
<td>9.1</td>
<td>157 ± 18</td>
<td>9554 ± 963</td>
<td>(.0001)</td>
<td>(.05)</td>
</tr>
<tr>
<td>Napindac</td>
<td>230</td>
<td>9.1</td>
<td>140 ± 9</td>
<td>9360 ± 739</td>
<td>(.0001)</td>
<td>(.01)</td>
</tr>
<tr>
<td>Napindac</td>
<td>61</td>
<td>9.1</td>
<td>nd</td>
<td>nd</td>
<td>110 ± 8§</td>
<td>527 ± 1363</td>
</tr>
<tr>
<td>Sulindac</td>
<td>123</td>
<td>9.1</td>
<td>171 ± 13</td>
<td>12 648 ± 1568</td>
<td>151 ± 15§</td>
<td>573 ± 751§</td>
</tr>
<tr>
<td>Sulindac</td>
<td>61</td>
<td>9.1</td>
<td>nd</td>
<td>nd</td>
<td>110 ± 8§</td>
<td>527 ± 1363</td>
</tr>
<tr>
<td>Sulindac</td>
<td>30</td>
<td>9.1</td>
<td>88 ± 6</td>
<td>(.80)</td>
<td>15 200 ± 3141</td>
<td>(.58)</td>
</tr>
</tbody>
</table>

*Seven-week-old mice were given a total dose of 9.1 mg of NNK in drinking water for 7 weeks (weeks 0 to +7) and NSAIDs for 9 weeks (weeks −2 to +7). Each A/J mouse received an intraperitoneal injection of sheep red blood cells (5 × 10⁷) 4 days before PFC assay. nd = not determined.
†Mean ± standard error.
‡Statistically different from control group by variance analysis (n = 10), three determinations each.

minutes. The numbers of cells secreting anti-sheep red blood cell IgM antibodies were determined in triplicate (41).

Preparation of splenocytes. Spleens were excised under aseptic conditions and placed in complete medium (RPMI-1640 medium, 1% bovine serum albumin, and penicillin-streptomycin at 10 IU/mL). Cell suspensions were obtained by teasing the spleen through 70-μm mesh in 4 mL of complete medium. Large debris were allowed to settle at room temperature for 5 minutes. The lymphocytes were collected by centrifugation through a Ficoll–Paque density gradient at 500g for 30 minutes at 25°C. At this stage, cells from five spleens were pooled to obtain a sufficient number of cells for all experimental conditions. Cells were washed twice in complete medium and depleted of monocytes by incubation in a sufficient number of cells for all experimental conditions. Cells were washed twice in complete medium and resuspended at 1×10⁶ cells/mL. Target populations. The nonadherent lymphocytes were removed, and the specific release of lactate dehydrogenase was measured in triplicate. Cytotoxicity of natural killer cells was calculated by standard procedures; spontaneous lactate dehydrogenase release by YAC-1 and natural killer cells was subtracted from experimental results. The optical density representing 100% cytotoxicity was determined by adding 2% Triton X-100 to YAC-1 cells (42).

Inhibition of PGE₂ synthesis. Blood (1 mL) was collected from the mice by cardiac puncture and stored briefly on ice in heparinized microtubes. The blood was centrifuged at 1500g at 4°C for 5 minutes, and the resulting plasma was stored frozen in liquid nitrogen until analyses. PGE₂ was extracted as described by Powell (43). A 10-μL aliquot of (20 000 disintegrations per minute) [5,6,8,11,12,14,15-3H(N)]PGE₂ (200.0 Ci/mmol) tracer was added to the samples to determine recovery. Each sample of plasma (200 μL) was vortex mixed with 2 mL of ethanol, incubated at 4°C for 5 minutes, and then centrifuged at 1500g at 4°C for 10 minutes to remove insoluble proteins. Pellets were washed with 2 mL of ethanol, and the supernatants were kept on ice. The combined supernatants were diluted with 4 volumes of 0.1 M phosphate buffer (pH 4.0). A C-18 reverse-phase cartridge (Waters Associates, Milford, MA) was rinsed with 5 mL of ultra-pure water and with 5 mL of high-performance liquid chromatography-grade hexane. PGE₂ was eluted with 5 mL of ethyl acetate containing 1% methanol. After evaporation of the samples to dryness, 0.5 mL of immunoassay buffer was added. Aliquots of 250 μL were used for scintillation counting, and 250-μL aliquots were used in a competitive PGE₂-monoclonal enzyme immunoassay according to the technique of Pradelles et al. (44).

Statistical analysis. Data were compared by variance analysis and Spearman’s test, followed by Student’s t test. Differences between samples were considered statistically significant at P < .05 (Student’s t test). In addition, the 95% confidence intervals were calculated by use of the computer program StatView (BrainPower, Inc., Calabasas, CA).

Results

NNK treatment. NSAIDs were given at nontoxic doses. NNK given at a cumulative dose of 9.07-9.11 mg/mouse during a 7-week period prevented normal body weight gain (Table 1). Food consumption by mice treated with NNK plus NSAIDs was not statistically different (P > .1) from food consumption by untreated mice or NNK-treated mice. When the mice were killed, no gross pathologic changes related to toxicity were observed in the liver, kidneys, stomach, intestine, or lungs of mice fed the diets with NNK plus NSAIDs.

Effects of NSAIDs on plaque-forming cell response. The primary humoral response to sheep red blood cells was determined in A/J mice treated with NSAIDs for 2 weeks. As shown in Table 2, plaque-forming cell response was expressed per million cells or per spleen. Reduction of plaque-forming cell response by NSAIDs was not statistically significant (P > .1).

Effects of NNK plus NSAIDs on plaque-forming cell response. The plaque-forming cell response was evaluated after 7 weeks of treatment with NNK alone or with acetylsalicylic acid, sulindac, or naproxen (Table 2). As expected, naproxen (230 mg/kg of diet), having no chemopreventive properties, was the only NSAID to reduce (by approximately 26%) the plaque-forming cell response expressed by a million cells or by spleen. Compared with the plaque-forming cell/spleen count of the NNK-treated mice (mean value = 14.704; 95% confidence interval [CI] = 11.304-18.103), that of the mice treated with NNK plus naproxen was reduced by 58% (mean value = 6187; 95% CI = 5464-6909) (P = .0007). The mean number of plaque-forming cells per million cells was 92
Effects of NNK and NSAIDs on plasma PGE\(_2\) level. NNK treatment more than doubled basal plasma levels of PGE\(_2\) (27.4 pg/mL; 95% CI = 22.1-32.4 pg/mL) compared with levels in untreated mice (60.4 pg/mL; 95% CI = 51.2-66.5 pg/mL) (Table 3). This increase was reduced by all the NSAIDs. Naproxen (230 mg/kg of diet) was the least effective (71% increase, \(P = .01\)), followed by acetylsalicylic acid at 254 mg/kg of diet (66% increase, \(P = .003\)). A nonlinear dose–response effect was observed for sulindac, with a return to basal levels of PGE\(_2\) in mice treated with the highest dose (123 mg/kg of diet). The mice treated with NNK plus NS-398 (7 mg/kg of diet) had a PGE\(_2\) level similar to that in untreated mice.

Discussion

Tobacco smoking suppresses humoral and cellular mediated immune responses in humans (32). Tobacco smoke contains numerous immunosuppressive agents, such as nicotine, benzo[a]pyrene, acetaldehyde, and N-nitrosodimethylamine (NDMA) (45-52). NNK, a nicotine-derived N-nitrosamine, is one of the most potent carcinogens found in tobacco smoke (36). A 4-month exposure to the structurally related nitrosamine NDMA suppresses plaque-forming cell and alloantigenic responses of T cells in CD-1 mice (50), and a 2-week exposure altered cell-mediated and antibody response to

### Table 3. Effects of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) with or without nonsteroidal anti-inflammatory drugs (NSAIDs) on plasma prostaglandin E\(_2\) (PGE\(_2\)) levels and cytotoxicity of mouse splenic natural killer cells against YAC-1 cells*  

<table>
<thead>
<tr>
<th>NSAIDs</th>
<th>NS-398</th>
<th>Naproxen</th>
<th>Acetylsalicylic acid</th>
<th>Sulindac</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg of diet</td>
<td>7</td>
<td>30</td>
<td>230</td>
<td>123</td>
<td>None</td>
</tr>
<tr>
<td>mg/mouse</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>% cytotoxicity, mean ± SE ((P))*</td>
<td>11.9 ± 1.0</td>
<td>7.9 ± 0.1(^*) (0.01/0.02)</td>
<td>4.0 ± 1.0(^*) (0.01/0.01)</td>
<td>4.5 ± 0.1(^*) (0.01/0.01)</td>
<td>11.9 ± 1.0</td>
</tr>
<tr>
<td>PGE(_2) levels, pg/mL, mean ± SE ((P))*</td>
<td>27.4 ± 5.5</td>
<td>65.4 ± 4.3(^*) (0.001)</td>
<td>45.5 ± 3.4(^*) (0.001/0.003)</td>
<td>47.0 ± 3.7(^*) (0.001/0.01)</td>
<td>65.4 ± 4.3(^*) (0.001)</td>
</tr>
</tbody>
</table>

*Seven-week-old mice were given a total dose of 9.1 mg of NNK in drinking water for 7 weeks (weeks 0 to +7) and NSAIDs for 9 weeks (weeks +2 to +7).
†YAC-1-to-natural killer cell ratio was 1.50. Mean ± standard error (SE).
‡P values from control group/NNK group. Statistically different from \(^*\)control group, \(^*\)control group (without NNK and NSAIDs), and \(^*\)NNK group (without NSAIDs); \(P\) values from two-sided Student’s \(t\) test; two groups of animals and three determinations per group for natural killer cell cytotoxicity; eight animals and four determinations per animal for PGE\(_2\) levels.
sheep red blood cells in (C57BL/6 × C3H)F1 mice (51,52). Immunosuppression of natural killer cell cytotoxicity by N-methyl-N-nitrosourea (MNU) has been observed (53). NNK, NDMA, and MNU have similar carcinogenic and DNA-alkylating properties (54). Our results suggest that NNK could contribute to the immunosuppression observed in smokers.

The first objective of our study was to determine the immunotoxicity of sustained exposure to NNK, which mimics long-term exposure of smokers to this carcinogen. We observed that a 7-week exposure of A/J mice to NNK inhibits primary humoral response to sheep red blood cells. Under these conditions, natural killer cell cytotoxicity against YAC-1 cells was reduced by 60%. In contrast, Lindemann and Park (48) observed no inhibition of cytotoxicity after a 4-hour exposure of natural killer cells to NNK, suggesting that NNK is immunosuppressive only after extended treatment. The lack of metabolic activation of NNK by natural killer cells could also be partially responsible for the absence of any observed inhibition (48). In a previous study (55), we observed no inhibition of plaque-forming cell response following a single injection of 5 mg of NNK into A/J mice.

We observed an elevation of the plasma level of PGE2 in NNK-treated mice bearing no detectable tumors. PGE2 functions are complex and incompletely understood. PGE2 is immunosuppressive, and elevated levels are observed in a variety of human and animal tumors (14). Reddy et al. (56) reported a twofold increase in PGE2 levels in rat colon tumors. We concluded that NNK immunosuppression is related to an elevation of PGE2.

Previous studies from our laboratory (24-26) have demonstrated the chemopreventive efficacy of NSAIDs against lung tumorigenesis, although the mechanism(s) remains to be elucidated. Bilo deau et al. (57) observed that sulindac and indomethacin inhibit PGE2 synthesis in A/J mice. Similarly, an attenuation of NNK-related elevation of PGE2 levels by NSAIDs was observed in our study. This extent of attenuation reflected the chemopreventive efficacies of NSAIDs observed in our previous studies (24-26). Acetylsalicylic acid and sulindac administration reduced the multiplicity of lung tumors in NNK-treated mice, whereas naproxen administration had no statistically significant effect (24-26). In this study, we have shown that plasma levels of PGE2 in mice were lower following sulindac or acetylsalicylic acid treatment than following naproxen treatment. Furthermore, sulindac treatment decreased PGE2 levels in the mice in a dose-dependent manner. The reduction of lung tumor multiplicity observed in sulindac-treated mice was also dose dependent (22,23). These results are consistent with those obtained by Reddy et al. (56) and Craven and DeRubertis (16), who observed that tumor PGE2 production was decreased considerably in aspirin-treated rats.

Acetylsalicylic acid and sulindac reduced NNK-induced suppression of plaque-forming cell response. In contrast, naproxen enhanced this immunosuppression. This was related to a slight, although not statistically significant, increase in lung tumor multiplicity observed in mice treated with NNK plus naproxen (25). Sulindac showed a dose–response relationship in the number of plaque-forming cells and plasma levels of PGE2, suggesting a relationship between these two parameters. Furthermore, the plaque-forming cell responses in sulindac-treated mice were inversely proportional to the number of lung tumors in A/J mice (Fig. 1). These results suggest that the protective effect of NSAIDs on the immune system parallel their chemopreventive properties. Likewise, acetylsalicylic acid attenuated the suppression of natural killer cell cytotoxicity induced by NNK, whereas naproxen had no effect (Table 3). Other investigators (58,59) have reported that natural killer function can be increased by indomethacin treatment and can be inhibited in vitro by the addition of prostaglandins. We hypothesize that the attenuation of NNK-induced inhibition of natural killer cell cytotoxicity may play a role in cancer chemoprevention by NSAIDs.

A new class of NSAIDs developed to treat acute and chronic inflammatory disorders selectively inhibits cyclooxygenase-2, the inducible isoform of this enzyme (60,61). The absence of ulcerogenic properties of this new class of NSAIDs is probably due to the maintenance of gastric prostaglandin synthesis by cyclooxygenase-1 (14,62). NS-398, the most studied member of this new generation of NSAIDs, was found to inhibit sheep placenta cyclooxygenase-2 with an IC50 (i.e., concentration that causes 50% inhibition of the enzyme activity) of 3.8 μM; however, cyclooxygenase-1 activity was observed to be completely unaffected at concentrations up to 100 μM (60). In our study, NS-398 was the most effective agent used against NNK-induced immunosuppression. Our results suggest that NS-398 could be very effective against chemically induced lung tumorigenesis.

Boraschi and Meltzer (63) observed in A/J mice a profound defect in the capacity of peritoneal macrophages to kill tumor cells over a wide range of experimental conditions. The number of plaque-forming cells and the percentage of natural killer cell cytotoxicity observed with A/J mice in our study were lower than those generally reported with other strains of mice (48-51). This defect in immunologic response could play a part in the susceptibility of A/J mice to lung tumorigenesis, as previously observed by Shimkin and Stoner (64). Our results demonstrate that the A/J strain is particularly suitable for the study of the efficacy of chemopreventive agents in preserving normal immune functions.

Our study provides some insights into the mechanisms of chemoprevention of pulmonary carcinogenesis. The inhibition of NNK-induced immunosuppression by specific NSAIDs and their effectiveness in inhibiting lung tumorigenesis suggest that the chemopreventive effects of these agents may be mediated through modulation of the immune system by PGE2 synthesis. Attenuation of inhibition of plaque-forming cell response and natural killer cell cytotoxicity, probably mediated by PGE2, could contribute to the prevention of lung tumorigenesis observed in mice treated with NSAIDs. These results do not exclude the possible implication of other mechanisms of chemoprevention by NSAIDs, such as induction of apoptosis (65).

References


Notes

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