Synergistic Antileukemic Effect of 6-Aminonicotinamide and 1,3-Bis(2-chloroethyl)-1-nitrosourea on L1210 Cells in Vitro and in Vivo

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ABSTRACT

A series of nicotinamide analogs were evaluated for their ability to inhibit L1210 cell poly(adenosine diphosphoribose) polymerase, and also for their ability to potentiate the cytotoxic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), nitrogen mustard, and γ-irradiation in L1210 cells growing in culture and in vivo. In vitro, nicotinamide, 5-methylnicotinamide, 6-aminonicotinamide, and benzamide effectively inhibited L1210 cell poly(adenosine diphosphoribose) polymerase; 1-methylnicotinamide, nicotinic acid, and benzoic acid did not. In culture, 6-aminonicotinamide potentiated the cytotoxic effect of BCNU; however, it did not significantly potentiate the effects of nitrogen mustard or γ-irradiation. In vivo, both 6-aminonicotinamide and nicotinamide potentiated the cytotoxic effect of BCNU; however, the concentrations of nicotinamide required for this effect were 10- to 20-fold higher than those of 6-aminonicotinamide. None of the analogs significantly potentiated the in vivo effect of nitrogen mustard or γ-irradiation. Treatment of L1210-bearing mice with varying combinations of BCNU and 6-aminonicotinamide produced a synergistic increase in life span; in some cases, the combination led to the production of long-term disease-free survivors.

INTRODUCTION

The chromatin-bound enzyme poly(ADP-ribose)3 polymerase responds to DNA damage by catalyzing the synthesis of poly(ADP-ribose) from NAD+ (11, 17). The polymer is covalently attached to chromosomal proteins and presumably modifies their structure and function so as to facilitate the repair of DNA strand breaks (4, 8, 19). Several analogs of the substrate NAD+ inhibit poly(ADP-ribose) polymerase, interfere with the DNA repair process, and potentiate the cytotoxic effects of DNA-damaging agents (4, 8, 16, 19). For example, Nduka et al. (16) showed that nontoxic concentrations of 5-methylnicotinamide potentiated the cytotoxicity of N-methyl-N-nitrosourea on L1210 leukemia cells as determined by soft-agar cloning assays. These observations suggest that poly(ADP-ribose) polymerase could be used as a target for cancer chemotherapy, and that selected analogs of NAD+ might be useful to potentiate the tumoricidal effects of cancer chemotherapeutic agents which act by inducing DNA damage. Smulson et al. (19) have in fact shown that nicotinamide causes slight potentiation of the antitumor effect of N-methyl-N-nitrosourea in mice bearing L1210 leukemia. While nicotinamide is a good inhibitor of poly(ADP-ribose) polymerase, it is also a normal metabolite, and its antitumor potential may be compromised by its utilization in normal metabolic pathways. In this study, we investigated a number of nicotinamide analogs for their efficacy as inhibitors of L1210 cell poly(ADP-ribose) polymerase, and for their ability to potentiate the antitumor effect of nitrogen mustard, BCNU, and γ-irradiation.

MATERIALS AND METHODS

Mice. Male BALB/c × DBA/2 F1 mice, hereafter called CD2F1, and DBA/2 mice, 6 to 7 weeks old, were obtained from Laboratory Supply Co., Indianapolis, Ind., housed in groups of 10 in plastic cages, and fed Purina laboratory chow ad libitum.

Tumor. L1210 leukemia cells were passed in male DBA mice at weekly intervals by i.v. injection of 0.4 to 1 × 106 cells/mouse. Tumor cell suspensions were prepared from the spleens of mice given injections the previous week, and cell counts were performed with a Model F Coulter Counter as previously described (9, 10).

Drugs and Reagents. Nicotinamide, nicotinic acid, 6-aminonicotinamide, 1-methylnicotinamide, and benzoic acid were purchased from Sigma Chemical Co. (St. Louis, Mo.). 2-Aminobenzamide, 3-aminobenzamide, and 4-aminobenzoic acid were purchased from Pfaltz and Bauer, Inc. (Stamford, Conn.). 5-Methyl nicotinamide was a gift from Dr. Kurt Gurzen of the Eli Lilly Research Laboratory (Indianapolis, Ind.). BCNU was supplied by the Division of Cancer Treatment, National Cancer Institute, NIH. Nitrogen mustard was obtained from Merck Sharp and Dohme (West Point, Pa.). Radiation was delivered with a Gamma Cell-1000, Atomic Energy of Canada, Ltd. [adenine-2,8-3H] NAD+ (specific activity, 3 Ci/mmole) was purchased from New England Nuclear (Boston, Mass.).

Enzyme Assays. Poly(ADP-ribose) polymerase activity was measured in L1210 cells rendered permeable to exogenously supplied nucleotides as previously described (1, 2). Briefly, L1210 cells were obtained from ascites fluid, diluted with phosphate-buffered saline, and then collected from suspension by centrifugation at 1000 × g for 10 min at 4°. They were rendered permeable to exogenously supplied nucleotides by a cold shock in a hypotonic buffer composed of 0.01 M Tris-HCl, pH 7.8, 20 mm 2-mercaptoethanol. The permeabilized cells were resuspended in the buffer at 2 × 10⁷ cells/ml, and 50 μl of the cell suspension were combined with 25 μl of reaction mix to give final concentrations of 33 mm Tris-HCl (pH 7.8), 20 mm 2-mercaptoethanol, 0.66 mm EDTA, 42.5 mm MgCl2, and 0.33 mm [adenine-2,8-3H]NAD+ (specific activity, 24 × 10⁶ dpm/mmol), and 1 × 10⁶ permeabilized cells (1, 2). Potential inhibitors were dissolved in 0.01 M Tris-HCl, adjusted to a final pH of 7.8, and 10-μl aliquots were added to the permeable cell assay system to test for inhibitory capacity. Reaction tubes were incubated at 30° for 30 min, and then reactions were terminated with an excess of cold 20%
trichloroacetic acid. Trichloroacetic acid precipitates were sonicated, collected on GF/C discs, extensively washed, and prepared for scintillation counting as previously detailed (1, 2).

Studies of Drug Interaction in Vitro. Male DBA mice were given 0.8 to 1 x 10^7 L1210 cells i.v. Four days later, 3 mice were sacrificed, and their spleens were removed, minced, and ground through a stainless steel screen in a modified Eagle's medium buffer with 3-(N-morpholino)propanesulfonic acid to pH 7.2 and supplemented with 10% fetal calf serum (9, 10). The cells were diluted with the same medium to a concentration of 6 x 10^5/ml and incubated with various nicotinamide analogs for 6 or 24 hr in plastic tissue culture tubes at 37° on a rotating wheel. Three hr after the incubation with the nicotinamide analog was started, BCNU or nitrogen mustard was added or irradiation was delivered to the cultures. Following incubation for 6 or 24 hr, cells were counted and 1 x 10^5 control or treated cells were injected i.v. into each mouse. As indicated in the appropriate legends, 5 or 10 mice were used to assay each treatment condition. Mice were inspected twice daily to determine the median life span of each group. The percent ILS due to the drug treatments was calculated as previously described (20) according to the following formula:

\[
\text{ILS} = \frac{\text{median survival treated animals} - \text{median survival untreated animals}}{\text{median survival untreated animals}} \times 100
\]

Study of Drug Interaction in Vivo. L1210 cell suspensions were prepared from the spleens of male DBA mice as described above, and 1 x 10^6 cells were injected i.v. into CDF2, mice (18 to 20 g). Two days later, drugs were given via the tail vein by direct i.v. push or by continuous infusion with a Harvard infusion pump. Each treatment group consisted of 5 animals, and all mice were inspected twice daily for 30 days to determine the median life span. Under these conditions, untreated mice live 8 to 9 days following the initial injection of L1210 cells. ILS due to drug therapy was determined by the formula given above. Mice that survived 30 days after treatment were considered to be long-term survivors and free of disease.

RESULTS

Table 1 compares the ability of a series of nicotinamide analogs to inhibit poly(ADP-ribose) polymerase in L1210 cells that were rendered permeable to exogenously supplied nucleotides. Since this assay is conducted in permeable cells, it specifically measures the ability of the agent to inhibit the enzyme without regard to cellular uptake. This assay gives no indication of whether the agents would be taken up by an intact cell. Nicotinamide was highly effective, giving 90% inhibition at a concentration of 2 mM. Analogs with additional substituents on the pyridine ring, such as 5-methylnicotinamide and 6-amino nicotinamide, were progressively less effective. The analog 1-methylnicotinamide, with a methyl group substituted at the ring nitrogen, had significantly less ability to inhibit poly(ADP-ribose) polymerase. Similarly, nicotinic acid, with a carboxyl group substituted for the carboxamide, was a poor inhibitor of the enzyme. Benzamide analogs were found to be highly effective enzyme inhibitors, 3-aminobenzamide being slightly more inhibitory than nicotinamide, and 2-aminobenzamide and 4-aminobenzamide somewhat less so. As with nicotinic acid, benzoic acid with a carboxyl group instead of the carboxamide showed little inhibitory capacity. These results are similar to those obtained with poly(ADP-ribose) polymerase from human sources (18) and suggest that agents found to be effective in the chemotherapy of L1210 leukemia may also be effective against human tumors.

Several of the nicotinamide analogs were evaluated for their ability to potentiate the effect of γ-irradiation, nitrogen mustard, or BCNU in L1210 cells. For these experiments, L1210 cells were treated in tissue culture and the effect of the treatment on cell viability was assayed by injecting the cells into mice and comparing their survival to those of mice given injections of the same number of untreated cells. As shown in Table 2, a 6-hr incubation of L1210 cells in nicotinamide had no significant effect on animal survival, nor did it potentiate the effect of γ-irradiation, nitrogen mustard, or BCNU. Results with 3-aminobenzamide and 5-methylnicotinamide were similar. In contrast,

### Table 1

<table>
<thead>
<tr>
<th>Agent</th>
<th>Poly(ADP-ribose) polymerase activity ( % of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide</td>
<td>91</td>
</tr>
<tr>
<td>5-Methylnicotinamide</td>
<td>88</td>
</tr>
<tr>
<td>6-Aminonicotinamide</td>
<td>70</td>
</tr>
<tr>
<td>1-Methyl nicotinamide</td>
<td>30</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>14</td>
</tr>
<tr>
<td>Benzamide</td>
<td>97</td>
</tr>
<tr>
<td>3-Aminobenzamide</td>
<td>96</td>
</tr>
<tr>
<td>2-Aminobenzamide</td>
<td>70</td>
</tr>
<tr>
<td>4-Aminobenzamide</td>
<td>73</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 2

Table 2

Effect of in vitro treatment of L1210 cells with nicotinamide analogs in combination with radiation and chemotherapy

L1210 cells were treated for 6 hr in tissue culture, then injected into mice as outlined in "Materials and Methods." Data are presented as percentage of ILS of mice receiving treated cells compared to those receiving untreated cells. The concentrations of nicotinamide, 3-aminobenzamide, and 5-methylnicotinamide presented in Column 1 were the highest that were evaluated.

<table>
<thead>
<tr>
<th>Analogs</th>
<th>Nicotinamide analog alone</th>
<th>300 R γ-radiation</th>
<th>Nitrogen mustard</th>
<th>BCNU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no analog)</td>
<td>0</td>
<td>21.3 ± 5.6</td>
<td>12.1 ± 6.4</td>
<td>27.3 ± 15</td>
</tr>
<tr>
<td>Nicotinamide (10 mM)</td>
<td>0</td>
<td>18.2 ± 12</td>
<td>8.2 ± 1</td>
<td>19.9 ± 4.4</td>
</tr>
<tr>
<td>3-Aminobenzamide (10 mM)</td>
<td>0</td>
<td>30.9 ± 9.7</td>
<td>47 ± 13</td>
<td>28 ± 1.4</td>
</tr>
<tr>
<td>5-Methylnicotinamide (5 mM)</td>
<td>3.8 ± 5.4</td>
<td>25.8 ± 3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Aminonicotinamide (1 mM)</td>
<td>21.7 ± 13</td>
<td>82.7 ± 20</td>
<td>76 ± 9.8</td>
<td>191 ± 58</td>
</tr>
</tbody>
</table>

Mean ± S.D. of at least 3 separate experiments, with 10 animals in each treatment group.
6-aminonicotinamide caused a 21% ILS and also potentiated the effects of γ-irradiation, nitrogen mustard, and BCNU. In each case, exposing the cells in culture to the combination produced a greater effect than the sum of the effects of the individual agents, indicating a synergistic interaction (14). No further potentiation of the effects of the radiation or chemotherapeutic agents was achieved by continuing the incubations for 24 hr.

We next evaluated the ability of 6-aminonicotinamide to potentiate the effects of increasing doses of radiation or chemotherapeutic agents. In these experiments, cells were exposed to the combination of agents in culture, and then 10⁷ cells were injected into each test animal. Chart 1 shows that 6-aminonicotinamide had an increasing synergistic effect with increasing doses of BCNU. While 6-aminonicotinamide increased the cytotoxic effects of nitrogen mustard, the degree of potentiation leveled off as the concentration increased. The effect of 6-aminonicotinamide on γ-irradiated cells was small and relatively constant over the range of radiation examined.

Since the combination of 6-aminonicotinamide and BCNU appeared to have the greatest synergistic effect on the cells treated in tissue culture, we selected this combination for further studies in vivo. Table 3 shows the results of our survey to determine the most effective way to combine these 2 agents for administration to L1210-bearing mice. Administration of 0.5 mg 6-aminonicotinamide by i.v. push or by 6- or 24-hr i.v. infusion produced a similar ILS and showed no difference in toxicity. Administration of 6-aminonicotinamide by i.v. push 3 hr before the i.v. administration of BCNU resulted in an additive effect for these 2 agents. Administration of 6-aminonicotinamide and BCNU by consecutive i.v. push gave an ILS of 51%, which indicates a synergistic effect of the 2 agents. The same ILS was obtained when the 6-aminonicotinamide was given by 6-hr infusion and the BCNU by i.v. push at the midpoint of the infusion. When the infusion was extended to 24 hr and the BCNU was given by i.v. push 3 hr into the infusion, there was essentially no enhancement of the BCNU effect. Thus, BCNU given simultaneously with 6-aminonicotinamide or at the midpoint of a 6-hr infusion of 6-aminonicotinamide, gave the greatest drug synergism. Since administration of the 2 agents by consecutive i.v. push was the simplest procedure and gave optimal results, it was selected for further testing. Chart 2 shows a more detailed examination of the schedule dependence for combining i.v. pushes of BCNU and 6-aminonicotinamide. The greatest ILS occurred when the 6-aminonicotinamide was given 30 min or immediately before the BCNU.

Table 4 shows a more detailed evaluation of the antileukemic interaction of BCNU and 6-aminonicotinamide in vivo. Increasing doses of 6-aminonicotinamide resulted in small increases in ILS; for example, at 0.7 mg/mouse, 6-aminonicotinamide produced a 25% ILS. At higher doses, 6-aminonicotinamide was toxic and resulted in premature death. Increasing doses of BCNU resulted in a progressive rise in ILS. At a dose of 0.4 mg BCNU per mouse, there was a 130% ILS but there were no long-term survivors. Treatment of the mice with 6-aminonicotinamide just prior to BCNU resulted in an enhancement of their survival. In general, the effect of the 2 agents was synergistic, since the ILS in L1210-bearing mice treated with the combinations was greater than the sums of the ILS obtained from mice treated with the individual agents. In addition, mice treated with 0.4 mg BCNU in combination with increasing doses of 6-aminonicotinamide showed 40 to 60% long-term survivors. This is in contrast to the failure to achieve any long-term survivors in mice treated with 0.4 mg BCNU alone. However, it should be noted that, at higher doses of 0.8 mg/mouse, BCNU is capable of producing long-term survivors. Similar studies in which nitrogen mustard and 6-aminonicotinamide were administered by direct i.v. push failed to show any synergism. Studies to evaluate the antileukemic interactions of increasing doses of BCNU with 3-aminobenzamide in the concentration range between 0.2 and 0.7 mg/mouse, or with nicotin-

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**Chart 1.** Potentiation of BCNU, nitrogen mustard, or γ-irradiation by 6-aminonicotinamide (6-AN). L1210 cells were incubated in culture for 6 hr with (O) or without (⊗) 1 μM 6-aminonicotinamide and exposed to the indicated concentrations of BCNU, nitrogen mustard (HN₂), or γ-irradiation. After treatment, 10⁷ cells were injected i.v. into 5 mice for each drug concentration, and ILS was determined relative to mice that received 10⁷ untreated cells. Points, mean values of experiments performed in duplicate.

**Table 3**

<table>
<thead>
<tr>
<th>6-Aminonicotinamide</th>
<th>BCNU</th>
<th>% of ILS⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. push</td>
<td>14 ± 8</td>
<td>26 ± 6</td>
</tr>
<tr>
<td>i.v. infusion, 6 hr</td>
<td>24 ± 7</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>i.v. push at 0 hr</td>
<td>51 ± 9</td>
<td>33³</td>
</tr>
<tr>
<td>i.v. push at 0 hr</td>
<td>52 ± 16</td>
<td></td>
</tr>
<tr>
<td>i.v. infusion, 24 hr</td>
<td>16 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

⁸ Mean ± S.D. of at least 2 separate experiments, with 10 mice in each group.

³ One experiment only.

**Chart 2.** Effect of different schedules on antileukemic activity of combinations of 6-aminonicotinamide (6-AN) plus BCNU. All drugs were given by direct i.v. push via the tail vein. All mice received 0.05 mg BCNU per mouse and 6-aminonicotinamide was given at a dose of 0.5 mg/mouse at the indicated times before or after BCNU ( ). Other mice received 0.05 mg per mouse BCNU alone (C), or 0.5 mg per mouse 6-aminonicotinamide alone (Δ). Values represent the mean of 3 separate experiments.
Table 4

Table 4

ILS of L1210-bearing mice due to combined effects of 6-aminonicotinamide and BCNU

L1210 cells (107) were injected i.v. into male CDF1 mice. Two days later, mice were treated with the indicated concentration and combination of drugs, each given by rapid sequential i.v. injection via the tail vein. Percentage of ILS was calculated relative to a 6-day survival in untreated mice. Numbers in Column 8 indicate the percentage of mice that survived for 30 days after treatment with 0.4 mg BCNU and the indicated concentration of 6-aminonicotinamide. In several cases where the observations were continued for prolonged periods, all mice which survived for 30 days were still alive after 60 days.

<table>
<thead>
<tr>
<th>6-Aminonicotinamide (mg/mouse)</th>
<th>BCNU mg/mouse</th>
<th>0.025 mg/mouse</th>
<th>0.05 mg/mouse</th>
<th>0.1 mg/mouse</th>
<th>0.2 mg/mouse</th>
<th>0.4 mg/mouse</th>
<th>0.4 mg/mouse survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/mouse</td>
<td>0</td>
<td>11.1 ± 9.6</td>
<td>13.9 ± 4.8</td>
<td>30.4 ± 12</td>
<td>74.9 ± 21</td>
<td>130 ± 26</td>
<td>0</td>
</tr>
<tr>
<td>0.2 mg/mouse</td>
<td>2.8 ± 4.7</td>
<td>19.5 ± 4.8</td>
<td>24.9 ± 8.2</td>
<td>77.6 ± 25</td>
<td>133 ± 43</td>
<td>322 ± 134</td>
<td>40</td>
</tr>
<tr>
<td>0.3 mg/mouse</td>
<td>2.8 ± 4.7</td>
<td>16.7 ± 8.4</td>
<td>38.9 ± 25.6</td>
<td>80.4 ± 29</td>
<td>138 ± 41</td>
<td>316 ± 104</td>
<td>40</td>
</tr>
<tr>
<td>0.4 mg/mouse</td>
<td>13.9 ± 4.8</td>
<td>17.6 ± 9.4</td>
<td>38.6 ± 9.8</td>
<td>83.4 ± 16</td>
<td>122 ± 19</td>
<td>339 ± 106</td>
<td>60</td>
</tr>
<tr>
<td>0.5 mg/mouse</td>
<td>13.9 ± 4.8</td>
<td>25 ± 16.7</td>
<td>50 ± 17</td>
<td>86.1 ± 20</td>
<td>147 ± 48</td>
<td>333 ± 115</td>
<td>40</td>
</tr>
<tr>
<td>0.6 mg/mouse</td>
<td>19.3 ± 9</td>
<td>41.6 ± 8.4</td>
<td>55.7 ± 19</td>
<td>78 ± 34</td>
<td>133 ± 35</td>
<td>400 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>0.7 mg/mouse</td>
<td>25.0 ± 14.4</td>
<td>47.2 ± 4.8</td>
<td>55.7 ± 19</td>
<td>89 ± 19</td>
<td>147 ± 26</td>
<td>400 ± 0</td>
<td>53</td>
</tr>
</tbody>
</table>

% of ILS after following doses of BCNU

a Mean ± S.D., based on 3 repeats of the experiment with 5 mice in each group. b Derived from a single experiment.

Antileukemic Effect of 6-Aminonicotinamide and BCNU

BCNU. L1210-bearing mice receiving BCNU alone showed an increase in survival which was proportional to the dose of BCNU. Treatment of the mice with BCNU plus 0.5 mg 6-aminonicotinamide resulted in prolongation of survival, with the greatest effect occurring at the highest doses of BCNU. There were no changes in the ILS or surviving fractions when the mice received concomitant treatments with BCNU plus 0.5 mg nicotinamide or 3-aminobenzamide per mouse; ILS and surviving fractions were the same as when they were treated with BCNU alone. Higher doses of 3-aminobenzamide could not be evaluated since they were toxic to the mice. Table 5 shows that higher doses of 5 to 10 mg per mouse of nicotinamide in combination with BCNU resulted in an increase in ILS and some long-term survivors.

Discussion

Poly(ADP-ribose) polymerase is a chromosomal enzyme that catalyzes the transfer of adenosine diphosphoribose moieties from NAD+ into poly(ADP-ribose) (11, 17). Treatments that cause DNA damage and DNA strand breaks stimulate the activity of poly(ADP-ribose) polymerase, and the time course of the increase in enzyme activity correlates with the processes of unscheduled DNA repair synthesis and the induction and repair of DNA strand breaks (4, 5, 8, 19). Studies of xeroderma pigmentosum cells reconstituted with Micrococcus luteus UV endonuclease demonstrate that poly(ADP-ribose) synthesis is required for restitution of normal DNA synthesis in UV-irradiated cells (4). Inhibitors of poly(ADP-ribose) polymerase impair the ability of the cells to rejoin DNA strand breaks and/or repair alkaline-labile DNA damage (8, 19) and enhance the cytotoxicity of DNA-damaging agents (16).

The studies outlined above serve as the basis for the proposal to use poly(ADP-ribose) polymerase as a target for cancer chemotherapy. Thus, if poly(ADP-ribose) synthesis is required as part of the DNA repair process, then inhibition of poly(ADP-ribose) polymerase should be useful to inhibit DNA repair and interfere with the ability of the cells to recover from damage induced by DNA-damaging agents. 6-Aminonicotinamide was found to inhibit poly(ADP-ribose) polymerase activity in permeabilized L1210 cells and to potentiate the effect of BCNU when intact cells were treated with combinations of the 2 agents. 6-Aminonicotinamide also showed a modest ability to potentiate the effects of nitrogen mustard and γ-irradiation. At the present time, it is not clear why this poly(ADP-ribose) polymerase inhibitor does not more significantly potentiate the effect of the other DNA-damaging agents. It is, however, noteworthy that other poly(ADP-ribose) polymerase inhibitors did not potentiate the effect of γ-irradiation in L1210 cells in tissue culture (16).

Some of the agents, such as 5-methylnicotinamide and 3-aminobenzamide, were good inhibitors of poly(ADP-ribose) polymerase in the permeable cell system but did not produce significant potentiation of the cytocidal effects of DNA-damaging agents. Although nicotinamide was found to potentiate the antileukemic effect of BCNU, high levels of this agent were required, which may be due to its utilization by normal metabolic pathways. For example, it has been shown that addition of nicotinamide to intact cells results in its rapid uptake and conversion to nicotinamide mononucleotide and then to NAD+ and NADP+ (3, 13). In addition, much of the nicotinamide is converted to 1-methylnicotinamide (13, 15) which, as shown in Table 1, eliminates its ability to inhibit poly(ADP-ribose) polym-
erase. 1-Methylnicotinamide is the form in which excess nicotinamide is excreted from cells (15), and it is possible that many of the nicotinamide analogs tested were rapidly methylated and excreted. Thus, another possible explanation for the failure of some of these analogs to potentiate the cytotoxic effect of the DNA-damaging agents may be that they are converted to inactive forms and eliminated from the cells and animals.

As yet, we have no direct information on the rate of methylation of 6-aminonicotinamide. However, the amino substituent at position 6 in this compound may produce steric hindrance to the enzymatic methylation at position 1, thereby accounting for the ability of 6-aminonicotinamide to potentiate the effect of the DNA-damaging agents in the intact cells. It is also possible that 6-aminonicotinamide acts by another mechanism. For example, it has previously been shown that 6-aminonicotinamide can be incorporated in place of nicotinamide into an NAD analog that could interfere with the oxidation-reduction capacity of the cell (7).

Previous studies have shown that 6-aminonicotinamide has limited antitumor activity when administered as a single agent (12). However, studies with this compound were abandoned when its toxicity became apparent and when many other chemotherapeutic agents were developed with much higher therapeutic indexes (6). Our present studies corroborate the observation that 6-aminonicotinamide has a small antitumor effect when administered as a single agent. We have shown, however, that it can be used at subtoxic doses to potentiate the effect of BCNU in a synergistic fashion. Further studies will be required to determine whether other inhibitors of poly(ADP-ribose) polymerase will be useful to potentiate the effects of other chemotherapeutic agents, and also to determine whether these combinations will be effective against other tumor types.

REFERENCES

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